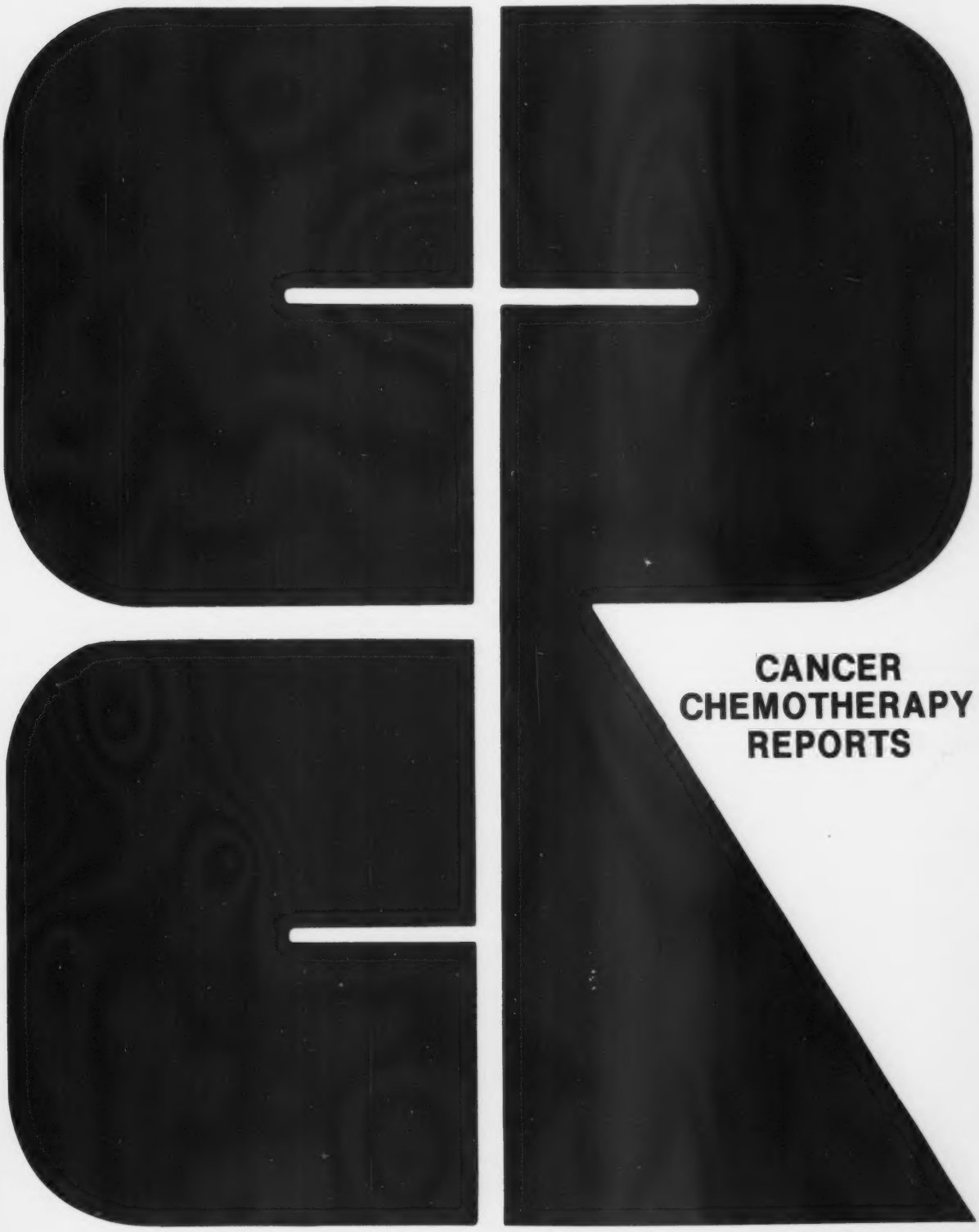

CANCER CHEMOTHERAPY REPORTS

Part 1

Sept/Oct 1975
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National Cancer Institute

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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

LETTERS

Myocardial Infarction in a 27-Year-Old Woman: Possible Complication of Treatment With VP-16-213 (NSC-141540), Mediastinal Irradiation, or Both[1]

VP-16-213 is a new semisynthetic podophyllotoxin derivative that is currently undergoing clinical testing (1,2). Although VP-16-213 toxicity has been reported to be mainly hematologic in nature, other toxic effects such as nausea, vomiting, alopecia, one questionable case of pancreatitis, and several cases of generalized allergic reactions have been noted (1,3). Three other side effects—hypotension occurring during rapid drug infusion, phlebitis, and anaphylaxis—have been reported in association with the administration of a closely related podophyllotoxin derivative, VM-26 (4,5),[2] but to date these effects have not been reported as having occurred during VP-16-213 administration.

Recently, we encountered an unusual complication that could possibly have been related to treatment with VP-16-213.

The patient was a 27-year-old woman who in 1967 (at the age of 19) was found to have clinical stage IIA nodular sclerosing Hodgkin's disease. From 1967 to 1972 she received four courses of radiation therapy to various areas above the diaphragm; two of these areas involved the mediastinum.

In October 1972, the patient was treated at the University of Arizona because of extension of Hodgkin's disease to her esophagus; further radiation therapy (to a dose of 3500 rads) was administered to the tumor-bearing area including the mediastinum.

In April 1973, chemotherapy with mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) (6) was started. Initially, the patient had satisfactory objective response to this program, but in August 1973, a superior vena cava compression syndrome developed, requiring additional radiation therapy to the upper mediastinum. Because of this progression of disease, chemotherapy with vinblastine, adriamycin, and bleomycin was started. When signs and symptoms of upper thoracic spinal cord compression developed, additional limited radiation therapy was given to this area. During this period the patient had evidence of uncontrolled, persistent Hodgkin's disease in the form of lymphadenopathy and bone pain in a rib lesion; and in June 1974, 5-day courses of treatment with VP-16-213 were started under the auspices of a Southwest Oncology Group phase II clinical trial. The initial 5-day course was started at a dose of 45 mg/m^2/day, infused intravenously (iv) over a 60-minute period. The patient tolerated the first course of treatment extremely well and subsequently received a second course at the same dose level, which was also well tolerated.

Nine days after the patient had completed her second course of VP-16-213 she was brought to the emergency room complaining of a heavy pressure sensation in her chest. She was found to be markedly hypotensive, and an ECG revealed changes compatible with an acute inferior and true posterior myocardial infarction, confirmed by enzyme studies.

During the patient's convalescence from her myocardial infarction there was evidence of persistently active Hodgkin's disease, and we decided to administer a third 5-day course of VP-16-213 at a dose level of 60 mg/m^2/day. The drug was again given by slow iv infusion over a 60-minute period. During the fourth daily infusion the patient suddenly became quite hypotensive (blood pressure, 60/40 mm Hg), and ECGs revealed new inversion of the T waves in the lateral precordial leads that was compatible with myocardial ischemia.

No further VP-16-213 was administered, and approximately 1 week later the patient was discharged from the hospital to continue convalescing from her myocardial infarction at home.

There are several possible explanations for the myocardial infarction in this patient including premature coronary artery disease, marantic endocarditis with emboli to the coronary arteries, cardiac toxicity from adriamycin, direct involvement of the heart by Hodgkin's disease, possible direct cardiotoxic (vascular) effects of VP-16-213, or possible potentiation of radiation-induced coronary artery disease by VP-16-213. Most of these explanations, such as involvement of the heart by Hodgkin's disease or marantic endocarditis (7), appear unlikely. Our patient did not have any of the known risk factors thought to be associated with premature atherosclerotic coronary artery disease. Adriamycin-induced heart disease usually occurs in patients who have received a cumulative dose of more than 550 mg/m^2 (8), and it is usually manifested by congestive heart failure or arrhythmias. Myocardial infarction has not been reported as a complication of adriamycin treatment. Furthermore, our patient received a total adriamycin dose of only 90 mg. Thus, it is quite likely that our patient's myocardial infarction was

[1]VP-16-213: 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside). Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute.

[2]VM-26: NSC-122819; 4′-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside).

due, in part, to either extensive mediastinal irradiation, direct VP-16-213 toxicity, or a combination of these two factors.

Mediastinal irradiation alone has been reported to induce coronary artery disease (9-11), and it is possible that the extensive prior mediastinal irradiation in our patient was solely responsible for her myocardial infarction. At present there is little or no clinical information on the cardiovascular effects of VP-16-213 and very little on the closely related podophyllotoxin derivative VM-26. Hypotension occurring during drug infusion has been observed with VM-26 (4), but it has not previously been reported for VP-16-213. (In one report, *hypertension* was related to the administration of VP-16-213 [2].) Neither delayed hypotension nor cardiac toxicity has been reported for either compound. In our patient, hypotension did develop suddenly during the last infusion of the third course of VP-16-213 and ECG changes consistent with myocardial ischemia were noted during this hypotensive episode. It is not known whether VP-16-213-induced hypotension might occur only during drug infusion or whether it could occur hours or even days later. In any case, hypotension occurring in a patient who already has compromised coronary circulation could precipitate a myocardial infarction. Furthermore, drug-induced coronary artery vasculitis could also contribute to myocardial ischemia.

Thus, further studies on the cardiovascular effects of the podophyllotoxin derivatives are clearly warranted. Until these studies have been completed, we urge caution in the administration of these agents to all patients who have had prior mediastinal irradiation or who have known coronary artery disease.

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Should the Diverse Biologic Activity of Effective Antitumor Agents be Considered a Hindrance to Their Clinical Use?

In a recent letter appearing in *Cancer Chemotherapy Reports*, Drs. Sieber and Adamson (1) expressed apprehensions concerning the experimental and clinical use of 1-methyl-1-nitrosourea,[1] a drug which has already found application for the treatment of patients with undifferentiated carcinoma of the lung and Hodgkin's disease (2). These apprehensions are based on their data in monkeys as well as the extensively documented data on the diverse biologic activity of this compound including mutagenic and carcinogenic properties.

Most of these data are quite familiar to us. In fact, the mutagenic activity of the alkylnitrosoureas was what attracted our attention to these compounds, leading to the systematic study of their antitumor properties. It should be noted that the derivatives of a strong mutagen of another type, ethylenimine, constitute one of the main groups of drugs used in cancer chemotherapy. We have succeeded in establishing the high antitumor activity of not only 1-methyl-1-nitrosourea but also of such mutagens as

[1] 1-Methyl-1-nitrosourea: NSC-23909; CAS reg. No. 684-93-5.

1-ethyl-, 1-propyl-, 1,3-dimethyl-, and 1,3-diethyl-1-nitrosourea, and also 1-methyl-1-nitrosobiuret (3,4). Moreover, it has recently been shown that another antitumor drug of this group, BCNU, also has mutagenic action and causes up to 35% mutations in Drosophila. A correlation probably exists between mutagenic properties and antitumor activity.

Several authors have noted that 1-methyl-1-nitrosourea causes chromosomal aberrations in normal and tumor cells in animals, in lymphocytes of the peripheral blood in man, and in embryonal fibroblasts in tissue culture. However, a study of chromosomal mutations in cultured peripheral blood lymphocytes of cancer patients treated with 1-methyl-1-nitrosourea has shown that the drug does not increase the frequency of cell aberrations in comparison with cells cultured before treatment (5).

Many nitroso compounds, including some of those mentioned above, are undoubtedly also carcinogens. This is also true for other antitumor agents, most of which are known to induce tumors in many different locations in experimental animals. Very often carcinogenic and carcinostatic activities are found in the same compound. This phenomenon, which was described by A. Haddow in 1947, is well known and other data were recently presented at the 11th International Cancer Congress in Florence in October 1974. It has also been reported (6) that the widely used active antitumor agent DTIC has carcinogenic activity in animals, inducing the development of breast cancer and lymphosarcoma in 100% of treated rats. The transplacental carcinogenic action of such well-known antitumor agents as procarbazine, 5-fluorouracil, and vinblastine, which induce breast cancer and tumors of the central and peripheral nervous system, is also described (7). In addition, the carcinogenic and mutagenic actions of the nitrofuran derivatives have been described (8). Finally, it is well known that radiation therapy has carcinogenic and mutagenic effects. Despite these observations, all of these therapies are widely used at present for the treatment of cancer patients. The problem is how to use these agents at dose levels and schedules which yield an objective response and yet do not display a carcinogenic action.

Treatment of advanced-disease patients with 1-methyl-1-nitrosourea results in a significant prolongation of lifespan, but such increases are still shorter than the time necessary for the development of a carcinogenic hazard. We have compiled data on more than 400 patients with different tumors who were treated with 1-methyl-1-nitrosourea and we did not observe new tumors in even a single case.

It should also be noted that according to our data, no new tumors were seen in rats with Sarcoma 45 treated successfully with 1-methyl-1-nitrosourea and observed to the end of their natural lifespans. Our attempts to induce tumors in these animals with 1-methyl-1-nitrosourea were not successful (unpublished observations).

With respect to the chemical properties responsible for the special difficulties with 1-methyl-1-nitrosourea, it should be mentioned that in our experience with the drug over more than 10 years we have not seen a single case of its spontaneous explosion. 1-Methyl-1-nitrosourea is sufficiently stable in solid state when it is stored in the refrigerator at 5°C, and it does not lose its activity over a 1-year period. The marked decomposition of 1-methyl-1-nitrosourea is known to take place only when the drug is stored for several months at temperatures of more than 20°C.

Aqueous solutions of 1-methyl-1-nitrosourea are, however, quite unstable. After storage of the drug solution for 1 day at room temperature its antitumor activity decreases by 10%-15% (unpublished observations at our Institute). However, this should not be an obstacle to the use of the drug because solutions of 1-methyl-1-nitrosourea, as well as those of many other drugs, should be prepared immediately before use. In addition, personnel handling such solutions should be extensively instructed to take the necessary precautions.

The current state of cancer chemotherapy does not allow us to consider the above-mentioned drug properties as contraindications for the clinical application of effective drugs, since such properties may result in a successful antitumor effect in patients with advanced tumors. Although 1-methyl-1-nitrosourea is quite effective when used alone, it is also a likely candidate for combined chemotherapy (9).

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April 28, 1975

Antitumor Activity of Calusterone (NSC-88536) in Advanced Breast Cancer[1]

In a recent issue of *Cancer Chemotherapy Reports* Falkson et al (1) reported extremely poor antitumor activity and severe side effects for calusterone combined with cyclophosphamide,[2] both given orally to menopausal patients with advanced breast cancer. Previously, Gordan et al (2,3) reported that 50 of 99 menopausal and postmenopausal women given calusterone had objective response. In a report of the Cooperative Breast Cancer Study Group (4), 28% of the women receiving calusterone compared with 18% of those receiving testolactone[3] for advanced breast cancer had objective remission in a randomized clinical trial. Additionally, the side effects were less frequent and severe in patients receiving calusterone.

In our trial only postmenopausal patients with disseminated and progressive breast cancer were studied. When patients had been given previous cytotoxic or hormonal treatment, a 1-month interval was requested before calusterone therapy was started. Calusterone was given at a dose level of 200 mg orally, divided into two doses during the two main meals. Three or more months of treatment was required before determining tumor response.

Thirty-one patients were treated with calusterone. Twenty-seven patients were considered evaluable for tumor response and four patients were not evaluable because treatment was discontinued due to severe gastrointestinal disorders. Eight patients (29.2%) who received calusterone had an objective response and two patients (7.3%) had a complete remission lasting 21 and more than 18 months respectively.

No further progression of the neoplastic disease was observed in eight patients (29.2%) who received calusterone for at least 3 consecutive months. The remaining patients (40%) had disease progression in spite of calusterone treatment. The majority of evaluable patients were more than 1 year postmenopausal and had a tumor-free interval of more than 1 year. Three patients less than 1 year postmenopausal failed to respond to therapy.

Hirsutism developed in 18 patients; it was mild in 14 and severe in four patients. Acne developed in 12 patients and in several patients it lasted a few months after treatment was discontinued. Gastrointestinal disorders accompanied by nausea were seen in ten patients. The symptomatolytic drugs were usually successful. No libido modification was observed. A significant rise of sulfobromophthalein retention occurred in about one third of the patients. Other laboratory values were not changed by treatment.

In conclusion, our study shows that calusterone is effective in the treatment of advanced breast cancer (primary and metastatic) with a moderate degree of toxicity. Our data agree with previous reports by the Cooperative Breast Cancer Study Group (4) and suggest a possible superiority of calusterone or other androgens yet to be used in the control of disseminated breast cancer. Finally, our data disagree with the results obtained by Falkson et al (1) who used a combination of calusterone and cyclophosphamide given orally. In contrast to our findings, they observed a very low antitumor activity and high degrees of toxicity, including neurologic.

[1]Calusterone: CAS reg. No. 17021-26-0; testosterone, 7β,17-dimethyl-. Supplied by Upjohn Italia.

[2]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[3]Testolactone: NSC-23759; CAS reg. No. 968-93-4.

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Metastatic Renal Adenocarcinoma Produced by Streptozotocin (NSC-85998)[1]

Previously we reported (1) on the renal tumorigenic action of streptozotocin in Holtzman rats but we hesitated to label some of the renal epithelial tumors as malignant since no metastases were found. However, in a male Holtzman rat, we have found a renal adenocarcinoma (fig 1) with metastases to the liver (fig 2), diaphragm (fig 3), mesenteric lymph node, and lower anterior pelvic subcutaneous tissue. Streptozotocin was given intravenously (iv) at a dose of 50 mg/kg × 1 and the animal was killed 463 days after treatment. In another rat treated with the same dose of streptozotocin and killed 465 days after treatment, two separate malignant neoplasms were observed: *(a)* a sarcoma of the kidneys with metastases to the liver, spleen, thymus, lung, mesenteric lymph node, adrenal, prostate, and colon; and *(b)* an adenocarcinoma of the kidney. These findings provide strong evidence that some of the renal adenocarcinomas that were previously reported may also have been malignant. In other studies on streptozotocin (2) no sarcomas (mesenchymal tumors) were encountered although a liposarcoma was observed in the lower pelvic region of a Holtzman rat treated with streptozotocin at a dose of 50 mg/kg × 1, iv and nicotinamide[2] at a dose of 350 mg/kg × 2 intraperitoneally (3). Since streptozotocin is widely accepted for the treatment of insulinomas (4), these findings take on added significance.

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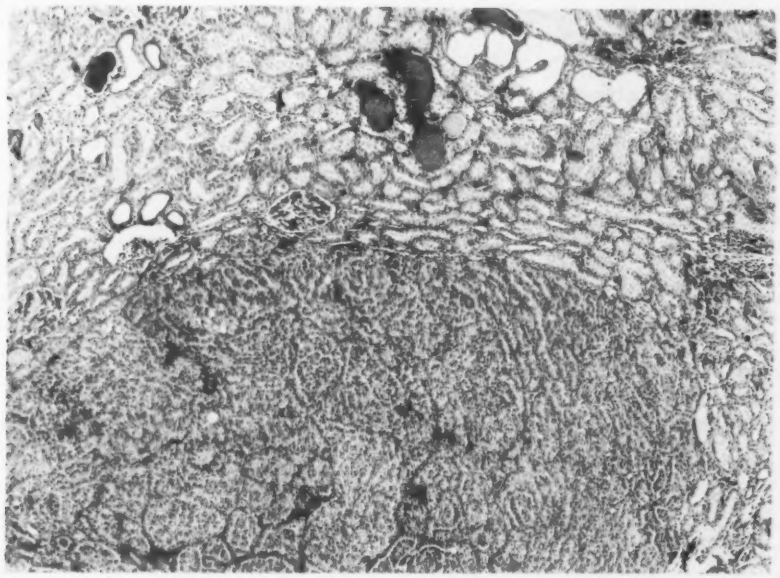

Figure 1.—Renal adenocarcinoma with areas of necrosis also showing renal medulla and compressed area of renal medulla (H & E stain, × 54).

[1]Supported by contract PH43-64-932 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[2]Nicotinamide: NSC-13128; CAS reg. No. 98-92-0.

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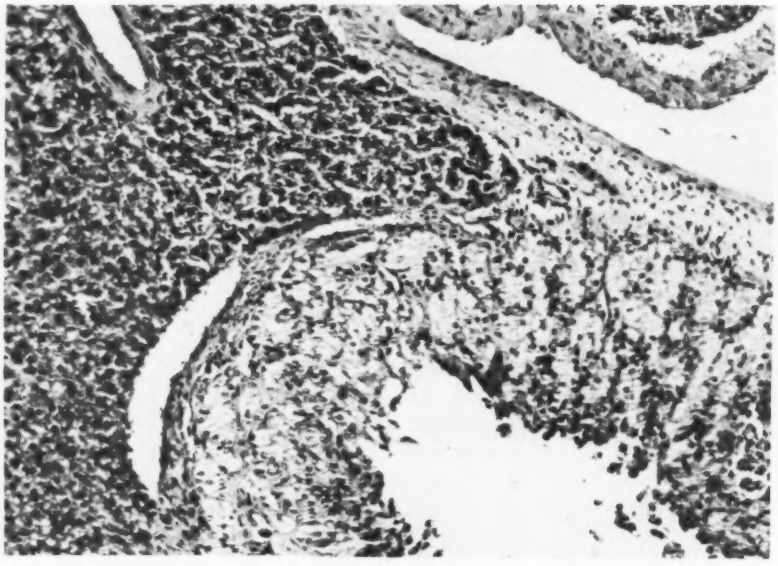

Figure 2.—Metastatic renal adenocarcinoma in liver (H & E stain, × 130).

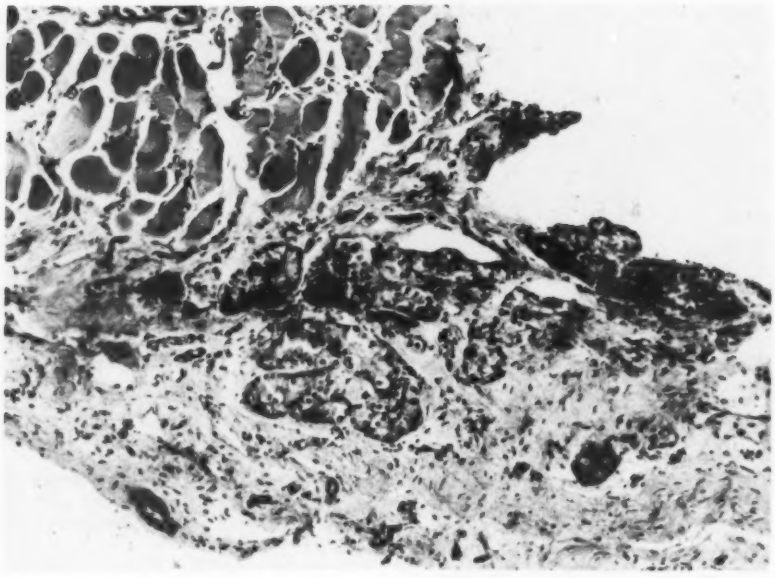

Figure 3.—Metastatic renal adenocarcinoma in lymphatics of diaphragm (H & E stain, × 130).

GUEST EDITORIAL

Combination Chemotherapy for Breast Cancer[1]

George P. Canellos[2,3]

The optimal management of breast cancer has not been established. This field has been confused by the multiplicity of effective but different therapeutic approaches. More recent controlled studies have suggested not only a limited role for extensive primary surgery, but also the lack of effectiveness of prophylactic endocrine ablation or radiotherapy (1). Patients with advanced disease refractory to hormonal manipulation were treated with cytotoxic drugs and it became apparent that a number of agents of differing pharmacologic action were active. These observations provided a reasonable basis for a combined drug approach. As with acute leukemia and lymphoma, the response to combination chemotherapy was considerably higher than the single-drug experience (2-5). In addition to a high overall response, the extent of tumor cell reduction appeared to be greater since complete clinical regressions were observed, an effect almost completely unknown with single-drug treatment (6-8).

There are now a proliferation of trials of various combination chemotherapy programs for advanced breast cancer. Each cooperative group or institution seems to have varied the dosage or deleted a drug to make its program just a bit different from the others (8). Thus, in a short while there will be great numbers appended to the complete response, partial response, and no response columns. There is, however, a very real likelihood that with critical analysis the overall response rates (even the complete remission rates) will not vary greatly from one study to the other. On the other hand, the toxic effects of these various programs may differ considerably depending on the type of patients included, the choice of agents, and the schedule of drug administration. An effective regimen which happens to be extremely toxic in patients with advanced disease should not be discounted for potential use as adjuvant therapy for patients in good nutritional state without metastases. The problem of toxicity from multiple-drug therapy cannot be underplayed, for it is inevitable that in the future useful drug programs will be applied to patients with stage II or even stage I disease. The potential intermediate or long-term toxic effects of new agents such as adriamycin and dibromodulcitol should be carefully considered.

To date, initial studies of combination chemotherapy seem to show a similar overall response rate regardless of the schedule employed. For example, the so-called "Cooper regimen" or slight modifications of it in large series have resulted in a consistent response rate of between 42% and 54% of similar duration. Smaller series from single institutions have slightly higher rates. Our pilot program at the National Cancer Institute (NCI), where cyclophosphamide and prednisone were given orally daily for 14 days every 28 days in addition to 5-fluorouracil (5-FU) and methotrexate given iv on Days 1 and 8 every 28 days (CMF plus prednisone), resulted in complete and partial responses in 64% of the patients with an 8-9-month duration of response (9). We have extended our experience with CMF plus prednisone and at the present time 20% of our patients have obtained a complete remission of all measurable disease. In contrast to the weekly drug administration of the "Cooper-type" program, we employ cyclic intensive therapy similar to the MOPP program with treatment discontinued for 14 days in each 28-day cycle. Because the overall results are similar to those of the "Cooper-type" regimens that include vincristine, the usefulness of that drug with its otherwise disagreeable side effects is questionable. Recent data from a prospective randomized study at the Mayo Clinic indicate that the addition of vincristine did not add to the response rate achieved with multiple drugs (10). In the Eastern Cooperative Oncology Group (ECOG), the CMF program has been randomly compared in previously untreated patients with intermittent melphalan. The response was superior in the CMF program (52%) compared to the melphalan program (24%) with a remission duration (8 months) that was in agreement with the duration previously seen in the NCI pilot study (11).

The precise role for adriamycin is yet to be defined. The drug is quite active as a single agent and in fact

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[1]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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may be the most active single drug for this disease. Its expense and toxic effects are considerable and there is a limited total dose that can be given (550 mg/m^2). Thus, its role might be confined to the induction of remission or in cyclic alternation with other programs. The Southwest Oncology Group study suggests that although the response rate is high, the complete remission rate is low and the duration of disease control is comparatively short (12).

The next obvious step is the application of combinations of drugs as adjuvants to primary therapy in patients with a high risk of relapse. The exciting but preliminary report of the adjuvant melphalan data jointly accumulated by National Surgical Adjuvant Breast Project, the ECOG, and the Central Oncology Group has prompted a sudden increase in studies using combinations for presumed early disease (13). The NCI of Milan has already begun a prospective randomized study comparing adjuvant CMF to no further treatment in 250 patients with positive axillary nodes. The study has been in progress for 18 months and the preliminary data show relapses in 12% of the untreated patients and in 2% of the CMF patients (14). In view of these findings, it is difficult to justify a placebo control for future adjuvant studies.

If one can extrapolate from the ECOG study which clearly shows a cytotoxic advantage of CMF over melphalan in advanced disease, then this combination or perhaps any of the others should be superior to melphalan.

With followup of such short duration, it is conceivable that melphalan is doing little more than delaying relapse. The rather modest antitumor effectiveness of melphalan in the ECOG study would suggest that only patients with small amounts of microscopic disease might benefit. At the very least the melphalan adjuvant study opened the door to adjuvant combination chemotherapy in a setting with a very real likelihood to make a tremendous impact on this common cancer. One can anticipate that in the future this concept of adjuvant therapy with single or combined drugs of high effectiveness against advanced disease will be applied to other tumors.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

CMF = cyclophosphamide, methotrexate, and 5-FU

Cooper regimen: cyclophosphamide, 5-FU, methotrexate, prednisone, and vincristine

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Dibromodulcitol: NSC-104800; CAS reg. No. 10318-26-0; galactitol, 1,6-dibromo-1,6-dideoxy-

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

MOPP = mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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COMMENTARY

Synergistic Interaction of Anticancer Agents: A Cellular Perspective[1,2,3]

Frederick Valeriote and Hsiu-san Lin[4,5]

The treatment of human neoplasms is increasingly being approached by the combined use of two or more anticancer agents. In addition, a widespread use of the terms "additive" and "synergistic" has developed to describe results obtained in both experimental and clinical studies. Since these terms produce different concepts in the minds of different investigators, there is a risk that certain results may be misunderstood and that incorrect inferences may be drawn in their transposition among investigators. Although extensive literature describing additivity and synergism of anticancer agents exists at the biochemical (1,2) and at the clinical (3) levels, there has been little discussion of these concepts at the cellular level. This is partly due to the lack of bioassay procedures for human tumor cells. In contrast to the clinical situation, it is possible to study the effect of anticancer agents at the cellular level in experimental animals. Both clonogenic (4-6) and lifespan increase assays (7,8) have been employed for transplanted and spontaneous tumors. Such studies have not only contributed greatly to an understanding of the mechanism of action of these drugs but also to the design of better treatment schedules.

We present in this paper an approach to the analysis of combination cancer chemotherapy at the cellular level and attempt to correlate this approach with data obtained from different experimental systems. We think it appropriate at this time to discuss and define the interaction of anticancer agents at the cellular level so as to provide a more rational basis for the combination of anticancer agents. Further, a better understanding of the cellular mode of action of the agents should help in directing study at the biochemical level and in the selection of those drugs and schedules most promising for producing additivity or synergism in the destruction of malignant cells.

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[2]Supported by Public Health Service grant CA-13053 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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Definitions of Synergism

To indicate the need for a redefinition of synergism at the cellular level, we need only examine the two main definitions presently used in cancer chemotherapy. First, Venditti and Goldin (7) state that therapeutic synergism occurs when a drug combination produces a therapeutic response, usually measured by an increase in lifespan (ILS) of tumor-bearing mice, that is superior to the maximum response achieved with either drug alone. As a recent example of this definition (9), the administration of 5-fluorouracil (5-FU) alone, at its optimal dose, produces a 100% ILS in L1210 tumor-bearing mice while methotrexate alone produces a 150% ILS; however, the combination of 5-FU and methotrexate at optimal drug levels produces a 200% ILS—a synergistic effect. On the other hand, Jawetz et al (10) define synergism as an excess of algebraic addition, as does Dorland's dictionary (11) where synergism is defined as the joint action of agents so that their combined effect is greater than the algebraic sum of their individual effects. The difficulty with this definition encountered by cell biologists, who use cell killing as an endpoint, can be illustrated by the example where one agent destroys 99% of the cell population and a second agent similarly destroys 99% of the cell population. A synergistic outcome from the combination of these two agents in terms of algebraic addition is meaningless.

That which implies synergism to one investigator can imply additivity or even subadditivity to a second investigator and can be meaningless to a third investigator. Because of this, we will define in the next section one meaning of synergism in cellular terms.

In dealing with anticancer agents, one must not only consider the dose of the agents but also the schedule of the agents, and while the effect of schedule modification has been shown to be quite important in combination chemotherapy we will exclude this factor from our discussion here. We will not be considering the combination of surgical treatment with either chemotherapy or radiotherapy nor of

either modality with immunotherapy. While a few specific examples of radiotherapy combined with chemotherapy will be given, the field is too wide for extensive analysis in this paper (12,13). Many of the concepts discussed here, however, are of such a basic nature that they can be appropriately extrapolated to these combinations.

Redefinition of Synergism

In discussing the interaction of anticancer agents at the cellular level, it is important to define the interactions in a quantitative manner. We will limit our discussion to one endpoint, that of cell survival. There is a fairly extensive literature which deals with the concepts of additivity and synergism both for antibacterial agents (10,14) and for other areas of pharmacology (15), and some of the underlying mechanisms discussed by these authors are pertinent to our discussion.

Let us examine the action of two anticancer agents, A and B. We will define the effect of each agent on a cell population in terms of cell killing, and discuss the different types of interactions that could occur when these agents are combined (fig 1). It should be noted that the definitions are not exclusive to malignant cell populations but are valid for any population of cells such as normal cells, bacteria, fungi, and protozoa.

Consider, first, the situation in which both agents have a similar effect on the cell population and reduce it to a surviving fraction of 10^{-1} (ie, 90% cell kill) (fig 1-I). If the survival fraction for the combination is greater than 10^{-1}, the term "antagonistic effect" would be used since the combined effect is less than that found for either agent alone. If the combination reduces the surviving fraction to between 10^{-1} and 10^{-2}, the effect would be termed "subadditive" as defined by Sartorelli (1); it is greater than that found for the most effective agent alone but less than additive. For a surviving fraction of 10^{-2} (ie, 99% cell kill), the combined effect would be termed "additive," being simply the product of the lethal effect of the separate agents.[6] When the agents

[6]The definition of the additive effect is the cornerstone for our other definitions. For the additive effect, it is assumed that each agent acts independently of the other and it is a consequence of probability theory that the product of the individual survival fractions is employed. It can be clearly seen why the older concept of algebraic summation is meaningless in this context, although it is meaningful if the parameter of ILS is assayed (16).

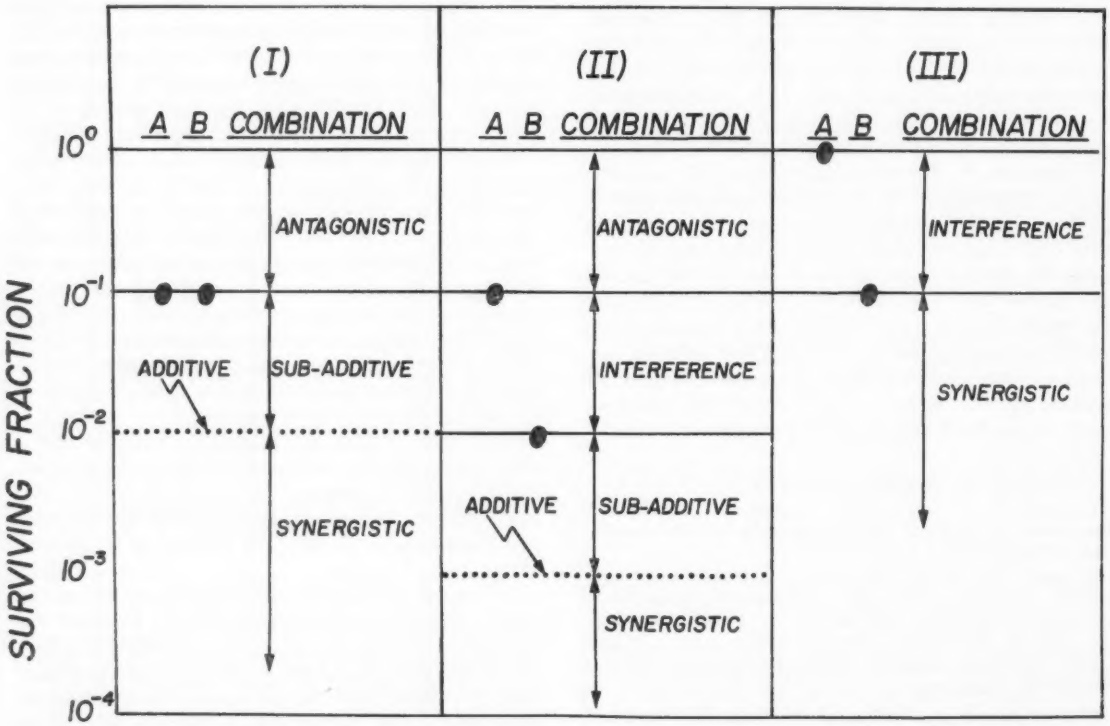

FIGURE 1.—Representation of the different results of drug combinations in terms of surviving fraction of the exposed cell population. The effect of the single agents alone is shown in columns A and B. Our definitions of possible outcomes of their combination are indicated.

result in surviving fractions of less than 10^{-2}, the effect would be termed "synergistic." While other terms such as enhancement, potentiation, and sensitization have been used to describe effects in which combined therapy proved to be better than single-agent therapy, they are generally employed when one of the agents has no activity for the effect being assessed. We will consider all of these terms to be equivalent to synergism.

Next, consider the situation in which the effects of the two agents are different. Assume that agent A yields a cell survival of 10^{-1} and agent B yields a cell survival of 10^{-2} (fig 1-II). If the surviving fraction for the combination is greater than 10^{-1}, the effect would be, termed antagonistic, since the combined effect is less than that found for the least effective agent. If the surviving fraction for the combination is between 10^{-1} and 10^{-2}, the term "interference" would be used because the effect is less than that found for the most effective agent alone. A surviving fraction of less than 10^{-2} but greater than 10^{-3} would be subadditive, 10^{-3} would be additive, and less than 10^{-3} would be synergistic.

Finally, consider the situation in which one of the agents has no effect on survival while the other agent results in a cell survival of, say, 10^{-1} (fig 1-III). Interference exists for a survival fraction greater than 10^{-1} and synergism exists for a survival fraction less than 10^{-1}. Classically, the latter effect has been defined as sensitization, as indicated earlier in this discussion.

When more than two agents are considered or when the agents are not given simultaneously, the above definitions still apply although the mechanisms of interaction are more complicated and difficult to interpret simply from survival data.

Cellular Mechanisms of Drug Interaction

Having defined possible interaction of anticancer agents in terms of cell survival, we will now examine a few biochemical and cellular mechanisms that could account for these different outcomes. This analysis will consider not only simultaneous drug administration but also the effects that are dependent upon an interval of time between the administration of the two agents.

1. The two agents could exhibit cytotoxicity as in figure 1-I or 1-II but have their effects on the same portion of the cell cycle. If both drugs kill 99% of the cells that are in the S phase of the cell cycle and if 50% of the cells are in this phase, then after the administration of one agent, the survival would be 50.5%. When the second agent is combined with the first, there would be an insignificant increase in cell killing and we would term this interaction as subadditive.

Even though the two agents act on the same portion of the cell cycle, an additive or even synergistic effect could be obtained if the second agent was given some period of time after the administration of the first agent (17). The optimum time interval would be equivalent to the time required for complete repopulation of the sensitive phase after administration of the first agent.

2. If the two agents acted at different phases of the cell cycle so that the first agent killed cells only in S phase and the second agent killed cells only in the G_2 phase, then, because they are independent of each other as to site of cytotoxicity, an additive effect on cell killing would be expected. It should be realized here, as in other examples to be discussed, that factors such as the pharmacokinetics of the drug or its kinetics of cytotoxicity can greatly modify the outcome of the drug interaction. For example, although one agent may kill cells only in the G_2 phase, it may remain exposed to the cell population for a period of time sufficient for a significant fraction of cells initially in the S phase to move into the G_2 phase and be killed. The cytotoxic action of the two agents would then not be independent. While these parameters of drug action are obviously important, we will not consider them further in this brief discussion.

3. In the event that the first agent not only kills cells but also blocks survivors from entering the phase of the cell cycle against which the second agent is effective, a synergistic outcome is possible. Agents such as hydroxyurea, methotrexate, and cytosine arabinoside have such blocking effects on cells entering DNA synthesis. Sometime after their administration, when the block has worn off, a large cohort of cells will synchronously move into the phase of the cell cycle for which the second agent demonstrates lethality and a greatly increased killing of cells could then be realized (18). Obviously, depending upon the time of administration of the second agent, an effect ranging from subadditive to synergistic could result.

4. One agent might block cells in their passage through the cell cycle and thereby lead to cell death by unbalanced growth. Prevention of this type of cell death can be achieved by inhibiting the remaining macromolecule synthetic activities which would result in interference. Synergistic cell killing has been demonstrated for situations in which substrates for DNA synthesis have been added in the presence of inhibitors of DNA synthesis (19).

5. The administration of one agent may not only kill a fraction of cells but also, as a consequence stimulate recruitment of resting (G_0) cells into the cell cycle. If the second agent was effective only in killing proliferative cells, it would be ineffective

on the original resting cell population but would become progressively more effective with increased recruitment. Thus, depending upon the time interval between the administration of the two agents, one could obtain subadditive, additive, or even synergistic cell killing. Such a protocol has been suggested by Schabel (20) with the administration of cytosine arabinoside and cyclophosphamide to a solid tumor.

While not intended to be an exhaustive list of possible modes of drug interaction which could be envisioned as leading to different survival outcomes, a few of the many obvious situations have been discussed to illustrate the interpretation of drug combinations at the cellular level. Few attempts have been made to define drug interactions in this manner at the cellular level (21).

6. There is a large variety of biochemical and pharmacologic effects that can be of relevance in the analysis of drug-combination effects on cell populations, a few of which are presented below.

A cell population can exhibit partial or complete resistance to a cytotoxic agent; this resistance might be overcome by a second drug which has no effect on the survival of the cell population when used alone but rather allows entrance of the cytotoxic agent. This effect has been demonstrated in the combination of the membrane-active polyene, amphotericin B (22), and the surface-active agent, Tween 80 (23), with anticancer agents. The synergism observed in these cases is due simply to increased drug uptake.

Other pharmacologic effects that can lead to an increased uptake of an anticancer agent include agents that either decrease the rate of catabolism of the second agent, increase its rate of activation, or decrease its rate of excretion (24). Included in this category are the radiation sensitizers such as actinomycin D and 5-bromodeoxyuridine, which can interfere with the repair of sublethal radiation damage through two different molecular mechanisms, and can result in synergistic cell killing upon subsequent irradiation.

As one example of interference, an agent could decrease the ability of a second cytotoxic agent to cross the cell membrane by competing for the same transport system, as has been shown for choline and mechlorethamine (25).

Interpretation of the Cellular Effect From the Whole-Animal Result

Finally we will consider more complicated examples where synergistic or additive effects thought to be occurring at the whole-animal level would not be considered as such at the cellular level. These examples will illustrate some of the problems of a cellular interpretation of effects observed in the whole animal.

First, consider the case where therapy is the optimal scheduling of either one or another agent alone and that in either case, 100% of the tumor-bearing hosts finally die despite this therapy. If the two drugs are given together, however, a significant fraction of the population is cured of the malignancy. Such an effect has been (appropriately) termed a synergistic effect at the clinical level. From a cellular point of view, it would be quite reasonable to suppose that neither agent alone cured the hosts due to the selection of a resistant tumor-cell population. By giving the drugs together, however, the population resistant to one drug was destroyed by the other drug and the probability of double resistance was so low that a significant number of hosts were cured of the tumor. If each drug alone resulted in a surviving fraction of 10^{-6} and if the host harbored 10^{10} malignant cells, then subadditive cell killing could yield this synergistic whole-animal result. A similar result could be expected even if we did not postulate the selection of resistant cell populations. The agents by themselves, even at their optimal schedule, could each yield a surviving fraction of 10^{-6} while in combination they could yield a cell survival level of less than 10^{-10}.

Second, consider the case in which malignant cells might exist in a protective compartment such as leukemic cells in the central nervous system (CNS) in advanced leukemia. In this case, two drugs could be given that, though ineffective in curing the host by themselves, cure a significant portion when given in combination. As in the previous situation, this would be termed synergistic at the clinical level, though it might not be so at the cellular level. For example, the host may harbor a cell population of 10^{12} cells of which 10^5 reside in the CNS. If we were to give one agent systemically that destroyed the malignant cells to a surviving level of 10^{-12}, the host would be cured of disease systemically; however, tumor cells might still remain in the CNS, recur systemically, proliferate, and kill the host. A second agent, able to pass the blood-brain barrier and yielding a cell survival of 10^{-7}, would destroy all leukemic cells in the CNS and destroy a similar fraction of the cells systemically. Cells would still exist systemically, however, and kill the host. If both agents were used together, even if they were subadditive in killing, all cells in the host could be destroyed, including those in the CNS, producing cures. This illustrates the case in which two agents that are additive or subadditive at the cellular level would appear synergistic at the whole-animal level. An experimental example of this situation has been demonstrated by Johnson

(26) with cyclophosphamide and radiation being given for systemic and CNS leukemia, respectively.

Any discussion at the whole-animal level brings us back to a consideration of the drug effect on the normal cell population and points out the importance of Goldin's "therapeutic" synergism. For example, a combination such as aminopterin and citrovorum factor can be scheduled so as to decrease the toxicity of the anticancer agent and thereby allow more of the drug to be given, increasing the destruction of malignant cells (27). While this would certainly not be considered synergistic from a cellular viewpoint it might be so considered by contemporary definition at the host level. In terms of host toxicity alone for drug combinations, an extensive study has recently been carried out by Schabel (28) with most of the combinations studied being less than additive or additive.

We have attempted to demonstrate that the terms used by investigators for synergism, by the clinician and experimentalist at the whole-animal level and by the cell biologist, generally are not translatable from one framework to the other with identical meaning or implication. Some of the conceptual difficulties have been described here and as more research in combination chemotherapy at the cellular level is forthcoming, many more examples will arise. Since the cancer therapist necessarily treats at the whole-animal level but conceptualizes at the biochemical and cellular levels, he must be aware of possible inconsistencies that may arise at the transition of one level to another.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053

Aminopterin: NSC-739; CAS reg. No. 54-62-6; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl]methyl]amino)benzoyl]-

5-Bromodeoxyuridine: NSC-38297; uridine, 5-bromo-2′-deoxy-

Citrovorum factor: NSC-3590; CAS reg. No. 6035-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-; folinic acid

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU

Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-; amethopterin

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COMMENTARY

Current Status of Clinical Immunotherapy

Avrum Z. Bluming

"It cannot be too often or too forcibly brought home to us that the hope of the profession is with the men who do its daily work in general practice. Our labors are in vain—all the manifold contributions of science, the incessant researches into the complex problems of life, normal and perverted, the profound and far-reaching conclusions of the thinkers and originators—all these are Nehushtan, sounding brass and tinkling cymbals, unless they result in making man better able to fight the battle against disease, better equipped for their ministry of healing" (1).

A rapidly growing, voluminous literature has developed around the concept of cancer immunotherapy. Extensive animal experimentation has documented the effectiveness of this modality and spawned a host of clinical trials interpreted as showing varying degrees of success. This article represents an attempt to review the rationale for this approach to the therapy of malignant disease and to critically assess its current value to the patients with cancer and its future promise for the clinical oncologist.

EXPERIMENTAL RATIONALE

As early as the late 19th century, investigators suspected that tumors possessed distinct antigens capable of eliciting host reactions. It was not until 75 years later, however, that experiments utilizing inbred strains of animals convincingly demonstrated that tumors, artificially induced with either potent oncogenic viruses (2-4) or chemical carcinogens (5-7), did indeed contain antigenic determinants specific for tumor tissue. In 1966, Mikulska and his colleagues reported experimental results strongly suggesting an immunologic mechanism for tumor rejection (8). Using the chemical carcinogen, benzo(a)pyrene, to induce fibrosarcomas in a syngeneic colonyof rats, these investigators tested the ability of isolated tumor cells to grow as autografts when injected back into the autochthonous hosts. The autografts were rejected if the primary tumor had been resected but grew in some animals in whom the primary tumor was left in place. In every instance the sarcoma cells grew when at least 2×10^6 cells were injected into previously unexposed syngeneic recipients. Mixing these tumor-producing cells with spleen cells taken from the tumor donor prevented or retarded tumor development in the injected, previously unexposed, syngeneic rats. This protective effect of autochthonous spleen cells was observed, however, only when the spleen cells were collected 3 weeks after excision of the tumor. When the spleen and tumor were removed from the donor rat at the same time, no protective effect was noted. These results suggest that the rats were capable of reacting against their own tumors, but that this reaction was a finite one that could be overwhelmed by the presence of a mass of tumor which, by adsorption, could exhaust the available supply of lymphocytes responsible for this protective reaction.

Abundant experimental data suggest that this protective reaction is mediated primarily by cells (9-12). Consider, for example, the following observations of Dr. Lloyd Law: the strongly antigenic polyoma virus is capable of inducing tumors when inoculated into certain strains of newborn mice. Although C57BL mice are normally resistant to the oncogenic effects of this virus, neonatal thymectomy nullifies this resistance; resistance can be restored by grafting syngeneic thymic tissue or by intravenous (iv) injection of syngeneic spleen or lymph node cells from nonthymectomized donors (13). Although susceptible to viral oncogenesis, the neonatally thymectomized mice are not totally anergic. Their response to weak antigens, such as the Y chromosome's histocompatibility antigen, is, indeed, eliminated, but they are still capable of rejecting potently antigenic allogeneic skin grafts. Thus, in this system moderate but not marked suppression of cellular immunity is a necessary precondition for overcoming resistance to tumor development.

Circulating antibody has also been shown to affect survival of transplanted tumors (14-16). In 1969, Hellström and Hellström (17) reported inhibited growth of Moloney sarcoma cells in culture when exposed to lymph node cells from mice in whom the

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virus-induced sarcoma had spontaneously regressed. However, serum from mice with active Moloney sarcomas blocked this lymphocyte-mediated inhibitory effect. The specificity of this blocking effect was demonstrated in two ways. First, serum from mice with sarcomas of different etiology did not block this lymphocyte-mediated inhibition. Second, the blocking effect of serum from mice with Moloney sarcoma could be neutralized by adsorption with Moloney sarcoma cells. The presence of blocking antibody was subsequently demonstrated in the sera of rabbits with Shope virus-induced papillomas (18) and in a spontaneous mouse mammary tumor system as well (19). This phenomenon of augmented tumor growth (termed immunologic enhancement) is felt to be the direct result of antibody protection of the tumor cells from lymphocyte attack (20-23).

This concept is further supported by the developmental nature of blocking activity in primary and grafted neoplasms studied. Blocking occurs more than 1 week before a tumor mass is palpable in most rats that develop primary neoplasms after neonatal polyoma virus infection (24,25). In this tumor system the titer of the blocking activity increases rapidly, reaches its maximum level when the tumor is detectable, and maintains this maximum level until the rats die (25). In rats receiving tumor isografts (from genetically identical rats), blocking activity generally appears 1 or 2 days before a tumor mass is palpable. In these animals as well, the titer increases rapidly, reaches its maximum soon after the tumor is first palpable, and remains at this level during the period of tumor growth. The blocking activity decreases to undetectable levels as early as 4 days following tumor excision in the rat polyoma system, becoming detectable again before or coincident with the recurrence of palpable tumor. The early appearance of blocking activity may thus play a critical role in the emergence of clinical tumor.

Enhanced tumor growth due to circulating antibodies may, however, be caused by activities other than simply the blocking of lymphocyte access to tumor cells. Antibodies directed against immunoglobulins or against lymphocytes are capable of inducing blast transformation and a concomitant increase in DNA synthesis by the target lymphocytes (26-29). Similarly, Shearer and his co-workers have recently demonstrated that a variety of murine and human cell lines are stimulated to reproduce and have increased longevity in culture following exposure to low titers of specific antibody (30). Significantly, antibodies that were stimulatory at low concentrations in this system were cytotoxic at high concentrations. This differential dose response to levels of circulating antibody has been confirmed in other laboratories (15). Finally, it is well to note that the blocking activity directed against the cell-mediated antitumor response may, in some instances, be due to circulating tumor antigen-antibody complexes and not to circulating antibody alone (31,32).

Several conclusions may be drawn from these experimental studies:

1. Autochthonous tumors, at least under experimental conditions, often do possess antigenic determinants recognizable by the host which can induce an effective rejection reaction.
2. The immunologic capability of the host is finite and this rejection reaction can be overwhelmed by tumor.
3. Moderate, not marked, suppression of cellular immunity is sufficient to suppress this rejection reaction.
4. Circulating antibody can play an active role in tumor rejection.
5. Circulating antigens, antibodies, or antigen-antibody complexes may interfere with cell-mediated rejection of tumor.

The ideal setting for immunologic rejection of tumor would thus be few tumor cells (either early or residual), containing antigens specific for the tumor, which are capable of eliciting a rejection reaction in a host with intact cellular immunity and no circulating blocking factors.

IMMUNOTHERAPY IN HUMAN CANCER

Having defined the theoretically ideal clinical conditions for tumor response to immunotherapy, we can review the real situations in which immunotherapy has been tried. The clinical manipulations which are included under the broad heading of immunotherapy are outlined in table 1.

Passive Immunotherapy

Passive immunotherapy involves transfusion of serum. The serum may be xenogeneic (from a member of a species other than man), allogeneic (from another human being who is not an identical twin), isogeneic (from an identical twin), or autologous (from the patient at some time in the past as, for example, during remission). In addition, the serum may be collected from untreated normal subjects (nonspecific) or from subjects vaccinated with tumor in an attempt to stimulate antitumor antibody induction (specific).

Nonspecific Passive Immunotherapy

Nonspecific passive immunotherapy is used every time a cancer patient receives a transfusion of whole blood or plasma. In 1951, Sir Lionel Whitby,

TABLE 1.—Modalities of immunotherapy

Mode	Nonspecific	Specific
Passive	Nonspecific or normal serum	Antitumor antiserum
Adoptive	Normal lymphoid cells	Sensitized lymphoid cells
Active	Nonspecific stimulants of the immune response	Tumor cell extracts, chemically modified tumor cells

then Regius Professor of Physic at Cambridge University, was able to conclude that "massive transfusion is now an established and recognized method of treating the acute leukemias" (33). This statement was based upon a review of numerous published reports of partial and complete remissions of acute leukemia following blood transfusion. In 1948, for example, Dreyfus culled a total of 21 such cases from the literature; in 14 of these patients remissions were reportedly complete and durable (34). Examples of these dramatic remissions include a 58-year-old woman with a 10-month survival after an 800-cc transfusion (35), a 49-year-old man free of leukemia 2½ years after a 780-cc transfusion (36), and a 19-year-old man without evidence of leukemia 20 months after a transfusion of 10 liters (37). In 1949, Bessis reported the results of exchange blood transfusion in 38 patients with acute leukemia, observing improvement in all patients and a complete remission in six (38). Exchange transfusion has subsequently been used in Australia to treat leukemic patients, but a complete remission was reported in only one of 48 patients so treated (39).

Aside from possible misdiagnoses, several explanations may be proposed to explain these occasional responses to transfusions of blood or plasma. The first relies on the traditional antigen-antibody responses proven operative in other diseases. Antisera produced in rabbits against an antigen present in the membranes of human acute leukemia cells (40-42) have been shown to be cytotoxic in vitro (41,42). In addition, cytotoxic antibody in normal human sera has been reported reactive with acute lymphoblastic leukemia cells (43). Serum from mice and humans containing C5 (the fifth component of complement) has marked antileukemic activity in leukemic AKR mice whereas C5-deficient serum has no such activity (16). It is thus possible that in humans the observed antileukemic effect of random blood or serum was due either to the presence of an unidentified antibody directed against the leukemic cells or to C5 present in the donor system.

An alternative explanation attributes the antileukemic effect of blood transfusion to killer lymphocytes in the transfused blood. This explanation is in accordance with Oren and Herberman's finding of lymphocyte reactivity against autologous leukemia cells (44) but does not explain Whitby's observation that the therapeutic result achieved with plasma is similar to that achieved with whole blood transfusion (33).

Finally, the explanation may not be immunologic at all, but may involve correction of a white blood cell maturation factor deficiency. In 1924 Sabin and associates reported in vivo maturation of human myeloblasts into myelocytes following the transfusion of whole blood (45). A similar phenomenon was observed by Schwind following transfusions of fresh plasma (46). Maturation of immature leukemic cells in vitro was reported as early as 1914 (47) and has since been reproduced in several laboratories (48-54). In 1929, Timofejewsky et al observed that normal plasma was required for in vitro leukemia cell maturation to occur (48), and in 1972 Fibach and his associates attributed this phenomenon to a differentiation-inducing protein believed to be present in normal plasma (54).

Specific Passive Immunotherapy

Highlights of the reported experience with specific passive immunotherapy are listed in table 2. Almost all of these studies are both uncontrolled and poorly detailed making impossible meaningful conclusions from the available data. Nevertheless, some of these studies are worth brief mention.

In 1901, Boeri reported objective regression in two melanoma patients treated with serum from a goat given repeated injections of cells from the specific melanomas (55). These regressions were partial and limited to several skin lesions. While the antitumor effect of the goat serum may have been real, waxing and waning of melanoma skin lesions is also seen occasionally in untreated patients.

Sumner and Foraker administered whole blood from a melanoma patient who underwent spontaneous remission to two untreated melanoma patients with active disease. One of the patients had a dramatic and long-lasting remission (56). Any causal effect of the transfusion on this patient's response may, of course, have been due to the administered cells rather than the serum.

In 1968, serum collected by Laszlo from normal volunteers immunized against normal allogeneic lymphocytes was administered to chronic lymphocytic leukemia patients. Three patients developed lymphopenia and a decrease in lymphadenopathy on this regimen. No effect was seen when serum from nonimmunized normal volunteers was used (57).

In 1967, both Burkitt in Uganda (58) and Ngu in Nigeria (59) reported objective regression of

TABLE 2.—Reported experience with different categories of immunotherapy

Year	Author	Ref No.	Tumor	No. of patients treated/ No. improved
Specific passive immunotherapy				
1901	Boeri	55	Melanoma	2/2
1960	Sumner and Foraker	56	Melanoma	2/1
1968	Laszlo et al	57	Chronic lymphocytic leukemia	3/3
1967	Burkitt	58	Burkitt's lymphoma	1/1
1967	Ngu	59	Burkitt's lymphoma	1/1
1969	Fass et al	60	Burkitt's lymphoma	8/0
1970	Bluming and Serpick	61	Burkitt's lymphoma	1/0
Nonspecific adoptive immunotherapy				
1963	Woodruff and Nolan	64	Cancer	8/8
1966	Schwarzenberg et al	65	Acute leukemia	21/9
Specific adoptive immunotherapy				
1967	Andrews et al	66	Melanoma, leukemia	4/0
1969	Nadler and Moore	67	Melanoma	53/13
1969	Trouillas and Lapras	68	Glioma	5/0
Specific active immunotherapy				
1902	von Leyden and Blumenthal	124	Cancer	?/0
1911	Risley	125	Cancer	20/0
1912	Coca et al	126	Cancer	79/1
1922	Kellock et al	127	Cancer	12/0
1951	Stone et al	128	Cancer	6/0
1960	Finney et al	129	Cancer	9/0
1962	Graham and Graham	130	Gynecologic cancer	232/0
1964	Aswaq et al	131	Cancer	21/0
1967	Czajkowski et al	132	Cancer	14/2
1969	Cunningham et al	133	Cancer	42/1
1970	Ikonopisov et al	134	Melanoma	13/0
1970	Hughes et al	135	Cancer	20/1
1970	Morton et al	97	Melanoma	18/3
1971	Marcove et al	136	Osteogenic sarcoma	15/6
1971	Krementz et al	137	Cancer	19/1
1972	Morton et al	138	Melanoma	6/0
Total for last group				526/15

Burkitt's lymphoma in a total of two patients treated with sera from patients in remission. A controlled trial of this form of therapy in eight patients was reported by Fass et al in 1969 (60); they observed no antitumor effect of the administered serum. In 1972, Bluming and Serpick plasmaphoresed a Burkitt's lymphoma patient in remission and returned several units of this serum to the patient at the time of relapse without clinical benefit (61). The discouraging results of this form of therapy should be viewed recognizing that although a minimal tumor burden insured optimal immunotherapeutic benefit in animal studies, disseminated tumor was present in all reported human trials.

Although passive immunotherapy alone has not been of significant clinical benefit to date, specific antitumor antisera, obtained by immunizing an animal to the tumor cells, are now being used as carriers of cytotoxic substances that will thereby be concentrated in and around tumor cells. In the first decade of this century, Ehrlich dreamed of using diphtheria toxin bound to antitumor antibodies as a magic bullet against malignant disease. Ghose and his colleagues in Nova Scotia have reported utilizing a chlorambucil-bound antitumor antibody to treat successfully the EL4 lymphoma in mice and one case of human cutaneous malignant melanoma (62). Similar studies have been reported in several experimental animal tumor systems. The cytotoxic substances employed in these studies include methotrexate, radioactive iodine, boron, diphtheria toxin, and the enzyme glucose oxidase. This unique application of specific passive immunotherapy has been concisely reviewed by Rubens (63).

Adoptive Immunotherapy

Adoptive immunotherapy involves administration of lymphoid or immunocompetent cells rather than

serum. These cells are believed to mediate the delayed hypersensitivity reaction, and are generally believed to be primarily responsible for transplantation immunity. Their specific immunocompetent ability may be adoptively transferred from one individual to another.

Nonspecific Adoptive Immunotherapy

Large numbers of immunocompetent cells transferred from one individual to another may induce a graft versus host reaction if the recipient does not reject the administered cells. A graft versus tumor reaction might also be expected, and this line of reasoning provides the rationale for the administration of large numbers of unsensitized lymphocytes as a form of anticancer therapy. The brief series of reported results of this nonspecific adoptive immunotherapy is listed in table 2. In 1963 Woodruff and Nolan (64) reported treating eight patients who had advanced cancer with iv infusion of all the cells from a normal human spleen. All the patients were pretreated with cytotoxic chemotherapy in an attempt to inhibit their ability to reject the donor's cells. Some improvement was reported in all eight cases, ranging from abolition of ascites to relief of metastasis-induced bone pain, but the relative contributions of the spleen cells and the prior chemotherapy remain conjectural.

In 1966 Schwarzenberg et al (65) reported treating 21 chemotherapy-resistant acute leukemia patients with 10^{12} leukocytes collected from patients with chronic myelocytic leukemia. Of the 21 patients treated, there were six complete and three partial remissions of short duration. Since the majority of cells administered were granulocytes and not lymphocytes, this interesting study does not properly belong in a series of adoptive immunotherapy trials.

Specific Adoptive Immunotherapy

The experience with specific adoptive immunotherapy (ie, the transfusion of lymphocytes specifically reactive against the tumor being treated) is summarized in table 2. In 1967, Andrews et al (66) reported cross-immunizing pairs of cancer patients with tumor tissue and subsequently exchanging their sensitized lymphocytes. Although they collected the lymphocytes by thoracic duct cannulation and were therefore able to administer a massive number of cells to each recipient, no therapeutic benefit was observed among the four patients so treated.

In 1969, Nadler and Moore (67) utilized a similar technique in a larger series of 53 patients. They collected the presumably sensitized lymphocytes from the peripheral blood, however, and reported 13 regressions of cutaneous malignant melanoma lesions, two of which were complete. In the same year, Trouillas et al (68) reported collecting autologous lymphocytes from patients autoimmunized against their own tumors. These lymphocytes, collected via the thoracic duct, were injected into the cerebrospinal fluid in a deliberate attempt to bypass the blood-brain barrier. Although no clinical benefit was observed following this therapy, post-mortem examination of the tumors disclosed uniformly massive lymphocytic infiltration.

Active Immunotherapy

Active immunotherapy relies upon the production of an antitumor reaction stimulated by injecting the cancer patient with either a nonspecific immune system stimulator or a specific tumor-antigen-containing preparation or both. This approach to treatment is based upon experimental proof that a growing tumor does not elicit a maximum antitumor response in the affected patient (69). The most frequently invoked presumption is that the autologous tumor antigen is not potent enough to induce an effective or cidal antitumor response. The application of nonspecific immunologic stimulation to the treatment of cancer is based upon this hypothesis and upon in vitro experiments suggesting that the cytotoxic activity of specifically sensitized lymphocytes occurs as a two-step process: *(a)* adherence of the lymphocytes to the target cells, a step that depends upon immunologic specificity; and *(b)* target cell destruction, the extent of which is determined in large part by the attacking lymphocytes' metabolic activity (69,70) which can be increased by lymphocyte stimulators such as phytohemagglutinin (69,71,72) and purified protein derivative (73). Stewart has hypothesized that in tumors demonstrating lymphocytic infiltration, believed to be a host antitumor response, nonspecific immunologic stimulation might increase the cytotoxicity of the sensitized lymphocytes directed against the tumor tissue (74). That such an effect could be produced in a mouse tumor system was demonstrated by Old and associates (75), who showed that nonspecific immunologic stimulation, using bacillus Calmette-Guerin (BCG), was capable, in some instances, of preventing the development of carcinogen-induced tumors (that is, tumors known to contain tumor-specific transplantation antigens). No such effect was noted in the case of tumors without defined tumor antigenicity, suggesting that this form of immunotherapy is effective only in tumors capable of inducing some immunologic response on the part of the host.

Nonspecific Active Immunotherapy

Infection in a patient with an aggressive malignant tumor is a serious, often fatal, complication.

Reports of spontaneous tumor regression following infection in such patients suggest that, contrary to expectation, severe infection may, on occasion, reverse the course of far-advanced neoplastic disease (76-81). Iatrogenic induction of infection in cancer patients in an attempt to reproduce this therapeutic effect has, on occasion, apparently succeeded (82-85). The most widely accepted explanation of this phenomenon proposes that these tumors are weakly antigenic in the host and that the infection, through reticuloendothelial stimulation, potentiates endogenous immunoreactivity directed against the tumor.

In 1969, Mathé et al (86) reported significant prolongation of remission from acute lymphoblastic leukemia after treatment with repetitive BCG vaccination. In 1971, a large-scale British study (87) failed to confirm Mathé's original results. In 1972, Bluming and his co-workers published a study designed to determine whether nonspecific immunologic stimulation could be demonstrated in malignant melanoma patients. After tumor resections half the subjects were vaccinated with the BCG preparation used by Mathé and half with that used by the British group on identical schedules. Assays of both circulating antibody and cellular immune reactivity to a battery of antigens were performed both before and after the course of repetitive vaccinations. Cellular reactivity to the antigens was significantly potentiated by the French vaccine while no such effect was noted following vaccination with the British preparation; neither vaccine potentiated circulating antibody reactivity. Although this study was not designed to determine therapeutic benefit, a marked difference in remission duration was observed (fig 1). All patients treated with the British BCG had evidence of recurrent tumor within 7½ months of surgery; in contrast, four of the six patients treated with the French BCG remained free of tumor after 24-32 months and one died of undetermined causes at 12 months, apparently still free of recurrent tumor (88).

The apparent difference in effect between these two preparations may well have been dose related (table 3), one being 10,000 times as large as the other. The more potent preparation stimulated cell-mediated reactivity and apparently prolonged remission duration. Although a dose-dependent effect is easy to understand, variation in BCG potency unrelated to dose poses a more difficult problem for the immunotherapist. A recently published comparison of ten different viable preparations of BCG, previously shown to be antigenically similar (89), disclosed a broad range of immunostimulant potential and therapeutic effectiveness (90). The best preparation for treatment of a given tumor, the optimum dose, and the most suitable mode of administration remain undetermined.

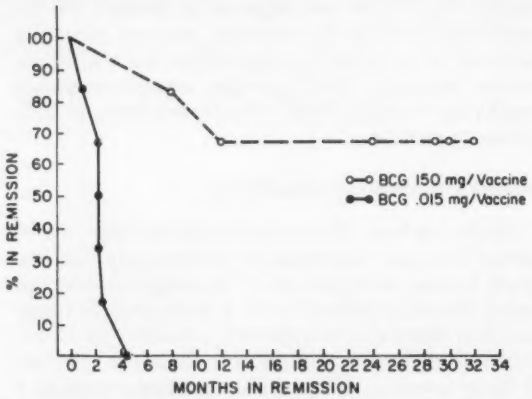

FIGURE 1.—Remission duration of melanoma patients treated with one of two BCG preparations (88).

This problem of selection notwithstanding, recently published studies have suggested that repetitive BCG vaccination is capable of prolonging remission duration in acute lymphoblastic leukemia (86), acute myeloblastic leukemia (91), and malignant melanoma (88,92) (table 4). All of Mathé's control patients had relapsed by Day 130 of remission, whereas almost half his BCG-treated patients were still in remission by Day 100 (86). Crowther et al (91) used irradiated allogeneic leukemic cells (a form of specific active immunotherapy) together with BCG. Eighty percent of their control patients and only 38% of their treated patients had relapsed at the time this study was published. In the study by Bluming and co-workers (88), patients were assigned to either low- or high-dose BCG based upon the degree of patient acceptance of the dermal scratching technique necessary for the latter. Although this study was therefore not randomized, the two groups of patients presented in figure 1 are comparable in terms of histologic subclassification of melanoma and anatomic staging. Gutterman et al (82) demonstrated a marked prolongation of remission duration associated with BCG treatment to patients following surgical excision of recurrent melanoma. In this study a BCG preparation from the Institute for Tuberculosis Research at Chicago (TICE strain) was found superior to the French strain already discussed. However, this effect may also have been dose related since approximately ten times as many viable organisms were administered with each TICE strain vaccine as with the French (Pasteur Institute) strain.

Minimal tumor cell burden is a precondition for successful immunotherapeutic trials. Since all patients in all series presented in table 4 were in complete remission, this criterion was therefore satis-

TABLE 3.—Preparations of BCG employed by Bluming et al (88) for treatment of malignant melanoma

BCG preparation	Wet weight concentration (mg/ml)	Volume administered (ml)	Dose administered (mg)
British (Glaxo)	0.15	0.1	0.015
French (Pasteur)	300	0.5	150

fied in distinct contrast to the studies discussed under the headings of passive and adoptive immunotherapy. Nevertheless, these data are not yet convincing. The remission duration of Mathé's control group is exceptionally short even for an unmaintained group of acute lymphoblastic leukemia patients (86); the difference between Crowther's two groups is not significant, although more recent studies by this same group (93-95) and one by Gutterman et al (96) support the initial impression of a favorable effect of BCG. Furthermore, Crowther's patients were treated with irradiated leukemic cells (91,95) and chemotherapy (93,94) in addition to BCG. The study by Bluming et al involved only 12 patients (88).

Although systemic BCG administration for nonspecific immunologic stimulation in cancer patients is still controversial, a related form of therapy, that of applying this preparation directly to a cutaneous tumor, is already of accepted benefit. The most dramatic and definitive demonstration of the antitumor activity of BCG in humans has been the regression of multiple intradermal metastases of malignant melanoma after intralesional injections of viable BCG. The original demonstration of this phenomenon by Morton and his colleagues (97) has been confirmed by a number of independent groups. In a recent review of the literature on this subject, Bast et al (98) found that cutaneous melanomas injected with BCG regressed in 58% of reported cases and, even more interesting, that in 14% of the cases, uninjected cutaneous lesions regressed concurrent with regression of injected lesions in the same patients. These results were observed only in those patients capable of mounting a cellular response to the injected BCG. In fact, in these reactive patients, close to 90% of BCG-injected lesions regressed; in contrast, tumor progression was the rule in those patients who remained tuberculin negative (97,99-101). The responses to intralesional injection have all been restricted to cutaneous lesions. To date there is no published evidence that human visceral melanoma regresses with topical BCG therapy. In animal studies, however, Baldwin and Pimm (102) have reported that pulmonary sarcomas, produced in rats by iv injection of antigenic sarcoma cells, were suppressed by BCG-sarcoma cell inocula at subcutaneous sites or by iv administration of BCG alone, and Zbar and co-workers (103) have published evidence suggesting regression of microscopic regional node metastases of an antigenic guinea pig hepatoma following intralesional injection of living BCG into intradermal tumor nodules.

Since human malignant melanoma is rarely limited to the skin, it is thus potentially curable with local application of BCG in only a minority of affected patients (approximately 10%-20%). An antitumor effect of topical or intralesional BCG has, however, been observed with a variety of other cutaneous tumors. Complete and long-lasting regressions of these tumors, including basal cell carcinoma, squamous cell carcinoma, and mycosis fungoides, have been reported by Klein et al (104,105), albeit often in conjunction with topical chemotherapy.

The potentially favorable therapeutic effects of BCG-induced nonspecific immunologic stimulation in the treatment of human cancer must be weighed against the reported toxic effects of this form of therapy (106). Repetitive intralesional injection of BCG frequently induces fever and malaise (98), a reported 24% incidence of hepatic dysfunction (107), a 12% incidence of granulomatous hepatitis responsive to antituberculous therapy (108), two cases of widespread, miliary, caseating granulomata (109, 110), two cases of shock resulting in death (111), and at least one case of enhanced tumor growth associated with the appearance of a circulating factor blocking specific cell-mediated antitumor immunity (112). Interestingly, no such serious complications have yet been observed following BCG application with dermal scratching in clinical trials involving several hundred patients (98).

A methanol-extraction residue (MER) of BCG has been shown by Weiss and his colleagues to protect experimental animals against nontuberculous microbial infection (113) and tumor isografts (114) probably through a process of nonspecific stimulation of the cellular immune response (115). Administration of this nonviable preparation for anticancer immunotherapy would avoid the infectious complications of BCG therapy already listed but would prevent neither the hypersensitivity reactions nor the danger of enhanced tumor growth (116). This latter complication was not observed, however, when MER was administered in association with either specific tumor antigen (116) or chemotherapy.[4] Widespread clinical trials of this extract in the treatment of human cancer are currently in progress.

Other agents currently under evaluation as nonspecific active immunostimulants include *Corynebacterium parvum*, the polyribonucleosides such as

[4]Weiss DW. Personal communication.

TABLE 4.—Experience with nonspecific active immunotherapy

Year	Author	Ref No.	Disease	Median remission duration (days)	
				Controls	BCG-treated patients
1969	Mathé et al	86	Acute lymphocytic leukemia	74	267
1973	Crowther et al	91	Acute myelocytic leukemia	210	280+
1972	Bluming et al	88	Melanoma	63	795+
1973	Gutterman et al	82	Melanoma	98	434+

polyinosinic-cytidylic acid (poly I-C) which have an immune stimulant activity in addition to interferon-inducing ability (117-119), and levamisole, an antihelmintic agent capable of potentiating cell-mediated immunologic reactions (120,121) and of facilitating chemotherapeutically induced remissions of murine leukemia (122). Clinical trials employing one or more of these agents as adjunctive therapy of minimal residual tumor are currently in progress.

Specific Active Immunotherapy

The experimental success of active immunotherapy may well be due to potentiation of the antitumor response against a weak tumor-associated antigen (TAA) as already noted. An alternative explanation, proposed by Alexander et al (123), is that the growing tumor may somehow block the regional lymph node from responding to the TAA. Alexander demonstrated a marked increase of stimulated thoracic duct lymphoblasts in mice following inoculation of tumor cells into a node-bearing area remote from that of the primary tumor. The clinical validity of this form of therapy is still unproven. The use of tumor cells for active immunotherapy has, nevertheless, been tried as treatment for a variety of human tumors as charted in table 2. The tumor cell preparation used for vaccination has been administered live (124,130), as a phenolized preparation (125) after irradiation (97,127,134,136,137), or following treatment with neuraminidase (138), an enzyme capable of splitting off sialic acid residues from the cell membrane thereby possibly exposing "new" antigens as immune stimulants (139,140). In an attempt to further potentiate the immunogenic capacity of these tumor preparations, they have on occasion been administered conjugated to carriers such as rabbit protein (132,133), BCG (97), pertussis vaccine (135), or complete Freund's adjuvant (135). The number of objective responses ("No. improved") shown in table 2 is less than spectacular. The great majority of these clinical studies were, however, performed on patients with large or disseminated tumors, a situation not conducive to successful experimental immunotherapy.

Transfer Factor

A leukocyte extract capable of transferring specific cell-mediated immune reactivity from one human to another was first described by Lawrence in 1949 (141) and was named transfer factor. Shown to be of clinical benefit in the management of patients with deficiencies of cellular reactivity such as the Wiskott-Aldrich syndrome and mucocutaneous candidiasis (142), it is currently under investigation as an immunotherapeutic agent in malignant melanoma, hypernephroma, and osteogenic sarcoma. Because transfer factor appears to be species specific, the only available source of this substance for cancer immunotherapy is from patients in long-term remission or from family members whose lymphocytes demonstrate cytotoxic capability against the tumor cells in vitro. Preliminary uncontrolled results of this therapy suggest some benefit and warrant continued study (142,143).

Immune RNA

In 1961 Fishman (144) incubated normal rat macrophages with a specific virus in vitro. RNA extracted from these macrophages was then added to normal lymph node cells and was found to confer upon these cells specific reactivity against the virus. Similarly prepared immune RNA was subsequently shown capable of transferring reactivity against transplantation antigens (145,146). Ramming and Pilch showed that immune RNA prepared from guinea pig macrophages that had been incubated with tumor could specifically immunize mice against the same tumor, demonstrating that, unlike transfer factor, immune RNA was not species specific (147-149).

In all these experiments the immune RNA incubated with autologous lymphoid cells produced newly

coded killer cells that were then reinfused into the animal donor to achieve specific antitumor immunity. The RNA was not injected directly into the host because it would have been inactivated by the high level of ribonuclease in the plasma and the interstitial fluid (138). If immune RNA were to be administered in conjunction with an effective ribonuclease inhibitor, this process might be circumvented and the immune RNA injected directly into the patient. Dextran sulfate inhibits ribonuclease and has a high molecular weight so that it is neither rapidly metabolized nor rapidly excreted. Administration of immune RNA together with dextran sulfate to experimental animals has already been shown capable of inducing specific antitumor immunity (150,151).

There are several theoretic advantages in the use of transfer factor or immune RNA as immunotherapeutic agents in the treatment of human cancer. Neither of these preparations is potently antigenic; host reaction against them, thereby curtailing their effectiveness, would be unlikely. These preparations transfer immunologic capability without the hazard of ensuing graft versus host reactions, and these agents should be effective even in individuals who are anergic because of defects in the afferent arm of their immune response. Finally, immune RNA, unlike transfer factor, may be effective even when obtained from a xenogeneic donor. Thus, immune RNA, active against human tumors, could be prepared from the macrophages of animals specifically immunized with the target tumor.

Thymus Extracts

Recently isolated extracts of bovine thymus have demonstrated the ability to increase the number and activity of circulating T lymphocytes (152-155). The feasibility of employing such an agent in the treatment of cancer patients is currently under active investigation (156,157).

CONCLUSION

This review of cancer immunotherapy has been based upon the hypothesis that the cell characterizing a specific human tumor is an autonomous enemy of the patient and that destruction of the last tumor cell is therefore the goal of therapy and would most surely result in cure. Serious reservations concerning this hypothesis have been raised, especially with regard to hematologic neoplasia, which several authors believe to represent either a defect in cell maturation (54), a disorder of leukopoietin regulation (158,159), a poorly controlled immunologic reaction (33,160-162), or a manifestation of antigenic viral infection (163). Interestingly, a beneficial effect of immunotherapy on the course of human leukemia or lymphoma is still awaiting confirmation. As regards treatment of nonhematologic malignancy, immunotherapy must still be regarded as a promising but unproven experimental modality, with the possible exception of its use in treating localized cutaneous tumors. Sir Alexander Haddow presented guidelines for the physician's approach to cancer immunotherapy in a 1971 address before the Royal Society of Medicine. In spite of some recent progress in this area, his comments at that time are equally valid today and would serve as appropriate concluding remarks for this review:

"In spite of a momentary over-optimism in certain quarters, it is probable that those who have made the greatest contributions to recent tumor immunology, and who possess the deepest knowledge of it, are perhaps the most cautious and least sanguine. Hence, the prospects of immunotherapy must be assayed with utmost balance, and our purpose must be, as in every other department of cancer research to stimulate both clinical interest and new experiments, while simultaneously repressing unjustified enthusiasm and prejudgement" (164).

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COMMENTARY

Cancer Chemotherapeutic Agents and Carcinogenesis[1]

Susan M. Sieber[2,3,4]

Recent advances in surgical treatment, nuclear medicine, and chemotherapy have led to significant increases in the survival of cancer patients with specific types of neoplasms; in some cases, such therapy has led to "cures," such as those seen in patients with choriocarcinoma, Burkitt's lymphoma, Hodgkin's disease, and Wilms' tumor. In addition, the immunosuppressive activity of several antitumor agents has been utilized to good advantage in some nonmalignant diseases, most specifically those categorized as "collagen-vascular" disorders. There is abundant evidence, however, that many antitumor agents cause structural and numeric damage to chromosomes, and that they are mutagenic, teratogenic, and carcinogenic in laboratory animals (1). Therefore, it is not surprising that some of these side effects are also seen in humans who are exposed to antitumor agents; fortunately, many of these effects appear to be of relatively minor importance.

Although the clinical sequelae of drug-induced chromosomal aberrations have not yet been well established, most clinicians are willing to risk cytogenetic damage in using cytotoxic agents to treat cancer. Similarly, some of the alkylating agents induce sterility in both men and women, but this is also considered to be an acceptable risk. Contraception is usually practiced by patients receiving cytotoxic drug therapy, thereby circumventing the possible teratogenic effects of these agents. Thus, the potential carcinogenicity of antitumor agents in man continues to be the most troublesome of the various side effects observed in laboratory animals. Whether or not clinically used antitumor agents do indeed possess this potential in man has not yet been demonstrated with certainty. Indirect evidence, based on the increasing number of reports of a second tumor arising in cancer patients during or after chemotherapy, is becoming available. Since many of these cases have appeared as isolated reports in the literature, it is difficult to estimate the frequency with which this phenomenon occurs. However, there are now a number of surveys of Hodgkin's disease and multiple myeloma patients that specify the total number of patients observed, the time period over which the observations were made, and the number of patients in whom a second neoplasm developed (which almost without exception has been acute myeloid leukemia [AML] or a variant of AML). From these surveys it has therefore been possible to make a crude estimate of the frequency with which this complication develops in patients with Hodgkin's disease and multiple myeloma.

The incidence of AML in the general population of the United States is 2.2 cases per 100,000 per year (2). For purposes of comparison, the estimated incidence of AML in nine groups of patients with Hodgkin's disease has been calculated from published reports and is expressed as cases per 100,000 per year. Figure 1 shows the incidence of AML in seven of these groups, plotted against the years over which the observations were made. The arrows in the figure indicate the approximate years that mechlorethamine and the mechlorethamine, vincristine, prednisone, and procarbazine (MOPP) regimen were introduced for the treatment of Hodgkin's disease.[5] In two series of patients with Hodgkin's disease that included over 3000 cases observed between 1944 and 1962, no cases of AML were noted (3,4). In five other groups of patients (5-9), the incidence ranged from 15 to 26 cases per 100,000 per year, representing a five- to ten-fold increase over the general population. The two highest values for incidence, 156 and 406 cases per 100,000 per year, came from relatively small groups of patients with Hodgkin's disease who were observed over relatively recent and narrow time intervals. The former group included 160 patients with Hodgkin's disease observed between 1961 and 1973 (10), and the latter group

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[5]Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin.

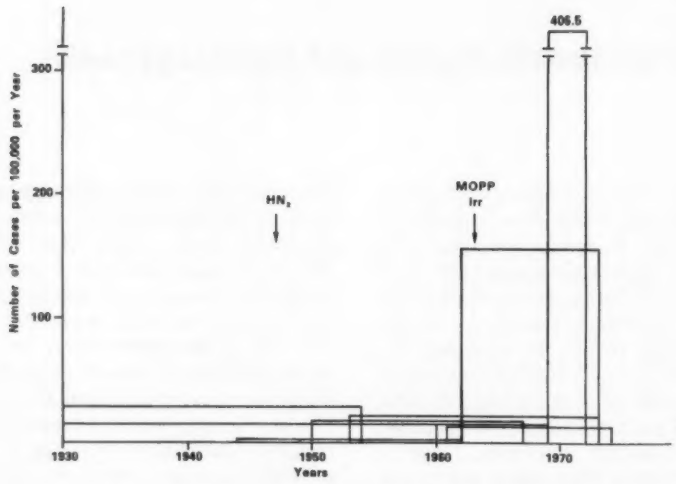

FIGURE 1.—Incidence of acute myeloid leukemia in patients with Hodgkin's disease.

consisted of 82 patients observed from 1970 until 1973 (11).

The incidence of AML, again expressed as cases per 100,000 per year, in seven groups of patients with multiple myeloma has also been calculated from published surveys (fig 2). In the earliest survey, no cases of AML were noted in 57 patients with myeloma who were observed between 1930 and 1956 (12). The next survey was published 8 years later (13) from which an incidence of 20 cases per 100,000 per year was estimated. In the four groups of patients with myeloma observed since 1963 (14-17), the incidence of AML has exceeded 500 cases per 100,000 per year, and thus represents a greater than 100-fold increase over the incidence of AML in the general population. The highest incidence, 5236 per 100,000 per year, was found within a small series of 19 patients observed by Andersen and Videbaek (14) between 1966 and 1970.

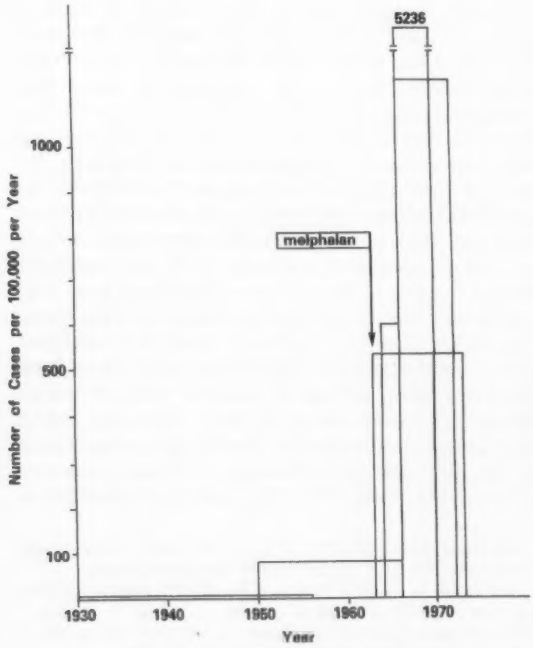

FIGURE 2.—Incidence of acute myeloid leukemia in patients with multiple myeloma.

On the basis of the available data, it is difficult to evaluate the role of cancer chemotherapy in this apparently increased incidence of AML in patients with Hodgkin's disease and multiple myeloma. There is some evidence that cancer patients are at a higher risk of developing a malignancy than is the general population, and it is possible that AML is part of the natural history of both Hodgkin's disease and multiple myeloma, which is only recently becoming evident because of the increasing numbers of long-term survivors of these diseases. Moreover, multiple myeloma and Hodgkin's disease are both associated with immune deficiency states: patients with multiple myeloma have low levels of normal immune globulins and respond poorly to antigenic stimuli (18), and untreated Hodgkin's disease patients possess defects in cell-mediated immunity (19). According to the "immunosurveillance" hypothesis, one of the

functions of the immune system is to recognize and destroy a cell or clone of cells with malignant potential that arises as a result of a somatic mutation (20). Thus, the inherent immune deficiency of myeloma or Hodgkin's disease may predispose these patients to malignancy.

The therapy for Hodgkin's disease and multiple myeloma, however, cannot be overlooked as a factor in the etiology of AML in the long-term survivors of these diseases. The leukemogenic effects of irradiation in man are well known, based on data derived both from the survivors of the atomic blasts at Hiroshima (21) and from a series of patients who received radiation therapy for ankylosing spondylitis (22). Therefore, irradiation may have contributed to the AML that occurred in Hodgkin's disease patients, since they all had received radiation therapy. Some other etiologic factor, however, must be considered for the AML found in patients with multiple myeloma, since only about one half of them had received radiotherapy. All of these patients had received cytotoxic chemotherapy, most frequently melphalan.[6] Although melphalan has immunosuppressive activity (23) and is carcinogenic in laboratory animals (24), it is difficult to extrapolate such animal data to the human. Many of the patients with multiple myeloma in whom AML developed after chemotherapy demonstrated a "preleukemic" phase characterized by bone marrow failure, pancytopenia, and sideroblastic anemia. A similar picture has been described for factory workers in whom acute leukemia developed after prolonged exposure to benzene (25); in fact, Cronkite (26) has stated that "any drug or chemical capable of inducing bone marrow damage must be assumed to be potentially a leukemogen."

Although patients with multiple myeloma, Hodgkin's disease, and possibly other cancers may be at risk of a second primary malignancy developing after they have received radiation therapy or chemotherapy, it is the concensus among clinicians that the benefits of chemotherapy in these conditions far outweigh any increased risk. The problem becomes more complex, however, when one contemplates the use of these agents in conditions in which their benefits have not been as clearly demonstrated. For example, protocols are being designed to determine the value of prophylactic melphalan administration subsequent to surgical treatment for stage I ovarian carcinoma. In addition, it has recently been reported that remission maintenance therapy with melphalan does not increase the median survival time of patients with myeloma, although it does increase the frequency of infectious complications (27). It may well be that these groups of patients are being exposed to the risk of drug-induced leukemia without deriving any demonstrable benefit from drug therapy.

Another group of patients consist of those treated with cytotoxic immunosuppressive agents for nonmalignant conditions (eg, kidney transplantation, chronic renal disease, systemic lupus erythematosus, psoriasis, rheumatoid arthritis, and the cold agglutinin syndrome), many of whom have a normal life expectancy. There are increasing numbers of cases in which a malignancy has developed in these patients after prolonged immunosuppression, most commonly with azathioprine.[7] In contrast to the AML in patients with Hodgkin's disease and multiple myeloma, the majority of neoplasms diagnosed in patients receiving immunosuppressive treatment have been lymphomas (28-31). The best documented population of such patients have been the kidney-transplant recipients (32). Hoover and Fraumeni have recently pointed out that after prolonged immunosuppression, kidney-transplant recipients are at a 30-fold higher risk of a lymphoma developing than is an age- and sex-matched population, and that most of this risk is due to a remarkably high incidence of reticulum cell sarcoma (33).

To date there is insufficient information as to the carcinogenic potential of antitumor or immunosuppressive agents in humans. A relatively large number of cancer patients have received chemotherapeutic agents and have benefited from such therapy. A second primary malignancy has developed in a fraction of these patients during or after chemotherapy, but an adequate statistical evaluation of this group is not yet possible because the "denominator," ie, the total number of patients treated with the agent in question, is rarely available. Also lacking are figures for the incidence of second tumors in cancer patients who have been randomly allocated to a regimen of therapy that does not involve antitumor agents. The recent adjuvant studies in breast cancer, comparing survival time after surgical treatment plus chemotherapy to survival time after surgical treatment alone, should provide this valuable information.

It is necessary, however, to know not only the number of cancer patients in whom a second primary malignancy develops after receiving chemotherapy, but also the size of the populations from which these patients come. To this end, a second-tumor registry should be established, fashioned after the Adverse Effects Registries of the FDA and AMA, or the

[6]Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[p-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-phenylalanine mustard.

[7]Azathioprine: NSC-39084; CAS reg. No. 446-86-6; purine, 6-[(1-methyl-4-nitroimidazol-5-yl)thio]-, monohydrate; Imuran.

Human Renal Transplant Registry organized by the American College of Surgeons. This registry, which might best be centrally located at the NCI, could store and evaluate data supplied both by private oncologists and by the 29 Cancer Centers which have been established across the country. It is only through a concerted cooperative effort on the part of the scientists, the statisticians, and the physicians who administer these highly active and highly toxic drugs that the question of their potential carcinogenicity in man will be resolved.

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Antitumor and Immunosuppressive Activities of Lankacidin-Group Antibiotics: Structure-Activity Relationships[1,2]

Koichiro Ootsu,[3] Takao Matsumoto,[3] Setsuo Harada,[4] and Toyokazu Kishi[4,5,6]

SUMMARY

The antitumor and immunosuppressive activities of the lankacidin-group antibiotics were studied in mice. Seventeen of 29 newly prepared lankacidin-group antibiotics, including 14-derivatives of lankacidin C, lankacidinol, isolankacidinol, and lankacidinol 14-acetate, possessed considerable antitumor activity against ascites 6C3HED/OG lymphosarcoma. Comparative studies on the antitumor activity of lankacidin C and eight of its derivatives against L1210 leukemia and solid 6C3HED/OG lymphosarcoma demonstrated that replacement of the hydroxyl group at position 8 or 14 of lankacidin C by an acyloxy group potentiated antitumor activity. However, these modifications of lankacidin C resulted in reduction of the immunosuppressive activity.

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Lankacidin-group antibiotics isolated from the fermentation filtrate of *Streptomyces rochei* var. *volubilis* (1-4) have a unique macrocyclic structure (fig 1) (1,5).[7] These antibiotics show strong antimicrobial activities, mainly against gram-positive bacteria including some strains of clinical isolates resistant to macrolide antibiotics (6), as well as weak toxicities in mice (7). Consequently, a series of lankacidin C derivatives were synthesized to evaluate the structure-antimicrobial activity relationships (7). The metabolic fate of the lankacidin C derivative 14-propionate has also been reported in a preceding paper by using ^{14}C-14-propionate (8).

Lankacidin C and its derivatives 8-propionate and 14-propionate were shown to possess considerable antitumor activity against L1210 leukemia, B16 melanoma, and 6C3HED/OG lymphosarcoma (9), and it was suggested that replacement of the hydroxy group at position 8 of lankacidin C by an acyloxy group might potentiate antitumor activity and reduce immunosuppressive activity. Based on these findings, a number of derivatives of lankacidin C were further synthesized to obtain more effective antitumor compounds.

This paper reports screening studies on 29 new lankacidin-group antibiotics modified mainly at position 8 or 14 of lankacidin C, including somewhat detailed studies on the activities of eight monoester derivatives against tumor growth and immune responses in mice.

MATERIALS AND METHODS

Compounds

The preparations and chemical characteristics of the lankacidin-group antibiotics used in this experiment have been described previously (3-5,7).[7] The positive control substances used were 6-mercaptopurine, cyclophosphamide, and L-asparaginase.

6-Mercaptopurine and lankacidin-group antibiotics were suspended in 0.2% acacia-physiologic saline solution. Cyclophosphamide and L-asparaginase were dissolved in physiologic saline solution immediately before being used. One-fifth ml of drug solution was administered intraperitoneally (ip) or orally to experimental animals.

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[2]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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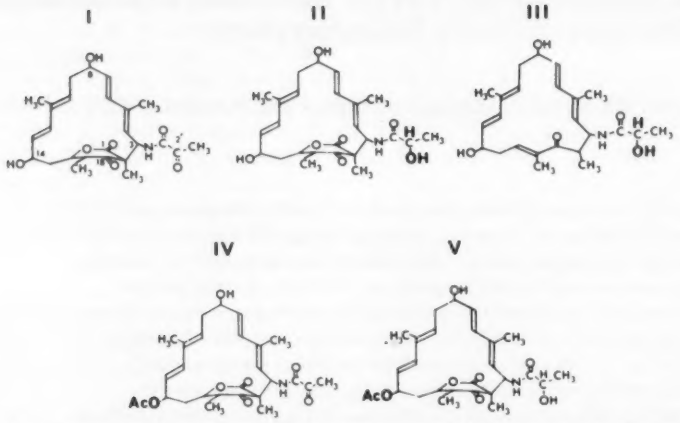

FIGURE 1.—Chemical structures of lankacidin-group antibiotics. I = Lankacidin C; II = lankacidinol; III = lankacyclinol; IV = lankacidin C 14-acetate; V = lankacidinol 14-acetate.

Animals

Specific pathogen-free C57BL/6, C3H/He, DBA/2, and hybrid (C57BL/6 × DBA/2)F_1 (BDF_1) mice of both sexes, weighing approximately 20 g, were obtained from the Drug Safety Center at this institution (9).

Tumors

One million L1210 leukemia cells (provided by the National Cancer Institute in 1971 and maintained in DBA/2 mice [9]) were transplanted either ip or subcutaneously (sc) into BDF_1 mice, and 10^7 6C3HED/OG lymphosarcoma cells (provided by the Aichi Cancer Center, Japan, in 1971 [9]), a variant sensitive to L-asparaginase, were also transplanted either ip or sc into C3H/He mice.

Evaluation of Antitumor Activity

Test drugs were administered ip or orally to mice daily for 5-9 days starting 24 hours after tumor implantation. To evaluate antitumor activity, the sizes of tumors or the survival times of tumor-bearing animals were compared in drug-treated and untreated groups (9-13). More than 25% increase in lifespan (% ILS) or more than 25% inhibition of tumor growth (% inhibition) in drug-treated groups compared to those in control groups was regarded as positive antitumor activity (12).

Primary Screening

Because of its high sensitivity to lankacidin-group antibiotics (9), an ascites 6C3HED/OG lymphosarcoma was used for our primary screening study. Dosages of 20, 25, 50, or 100 mg/kg of the antibiotics were selected for evaluating comparative antitumor activity based on the findings that lankacidin C given at a dose range of 20-100 mg/kg caused considerable prolongation of the survival time of tumor-bearing mice; at this dose range, 67%-100% of the drug-treated mice survived for more than 30 days after tumor transplantation whereas all control mice died within 20 days (9). A survival rate of more than 60% in the drug-treated group 30 days after tumor transplantation was regarded as evidence of antitumor activity (9).

Antibody Production

Sheep erythrocytes kept in Alsever's solution (Shiihashi Co., Tokyo, Japan) were washed aseptically three times with physiologic saline solution and adjusted to 5×10^8 cells/ml. A total of 0.2 ml of the suspension was then injected intravenously (iv) into C57BL/6 mice. The mice were bled 5 days after the immunization and the microhemagglutinin titer of the serum was tested by a standard method (12). The highest serum dilution showing a definite agglutination of the erythrocytes was taken as the reciprocal antibody titer.

Test drugs were administered ip or orally to mice for 4 consecutive days starting 24 hours after immunization. Reduction of the antibody titer in the drug-treated group to less than one fourth of the level in the untreated group was regarded as positive immunosuppressive activity (12).

Graft-Versus-Host Reaction (GVHR)

The GVHR (14) was conducted in adult mice according to the procedure of Gray et al (15) with a minor modification (12). BDF_1 recipient mice (5 weeks old) were injected iv with 4×10^7 spleen cells from 8-week-old C57BL/6 donors. Test drugs were administered ip to mice injected with spleen cells (three mice/group) or to uninjected mice (three mice/group) for 5 consecutive days from 24 hours after injection of spleen cells. Five days after the last drug injection, the weight of the spleen and body weights of the recipient mice were measured. The GVHR index was calculated from the following equation (15):

$$\text{GVHR index} = \frac{\text{mean spleen weight of spleen cell-transferred mice}}{\text{mean spleen weight of spleen cell-untransferred mice}}.$$

The suppressive activities of the test drugs were demonstrated by comparison of the GVHR indices of drug-treated and untreated control groups as follows:

$$\text{Inhibition rate (\%)} = \left(1 - \frac{\text{GVHR index of drug-treated group} - 1}{\text{GVHR index of drug-untreated control group} - 1}\right) \times 100.$$

Inhibition rates of more than 60% were regarded as indicating positive immunosuppressive activity (12).

RESULTS

Primary Screening

Ascites 6C3HED/OG Lymphosarcoma

The results of preliminary experiments on the antitumor activities of 32 lankacidin-group antibiotics against ascites 6C3HED/OG lymphosarcoma are summarized in tables 1-3. In these experiments, an ip dose of 20 or 100 mg/kg was given for 5 successive days, since the former dose was the minimal effective dose of lankacidin C against the tumor (67% of the drug-treated mice were alive 30 days after tumor transplantation) and the latter dose was a sufficient dose for confirming its activity (all drug-treated mice survived) (9). An ip dose of 25, 50, or 400 mg/kg of some lankacidin C derivatives was also used to confirm their antitumor activity. No signs of toxicity were observed after administration of these doses.

Five of eight derivatives modified at position 8 of lankacidin C, 8-acetate, 8-propionate, 8-butyrate, 8-valerate, and 8-caprate, were effective in prolonging the survival time of tumor-bearing mice at a daily dose of 20 mg/kg. Two other derivatives, iso-butyrate and iso-valerate, were ineffective at a dose of 20 mg/kg (table 1). However, in another experiment, these antibiotics were significantly effective at a daily dose of 100 mg/kg causing a 100% survival rate for tumor-bearing mice. The palmitate derivative of lankacidin C was inactive even at a daily dose of 400 mg/kg (table 1).

Four of eight derivatives modified at position 14 of lankacidin C, 14-acetate, 14-propionate, 14-iso-butyrate, and 14-caprate, showed antitumor activity at a daily dose of 20 or 25 mg/kg (table 2). The lankacidins 14-butyrate, 14-valerate, and 14-iso-valerate were ineffective at a daily dose of 20 mg/kg, but in another experiment these antibiotics were active at a dose of 100 mg/kg, causing a 100% survival rate after 30 days. The palmitate derivative of lankacidin C showed no antitumor activity even at a daily dose of 400 mg/kg (table 2).

Four of 15 other derivatives, lankacidin C-8,14-diacetate, lankacidin C-8,14-dibutyrate, and lankacidinol and its 14-acetate derivative, were effective in prolonging the survival time of tumor-bearing mice at a daily dose of 100 mg/kg (table 3). Among these, lankacidinol and its 14-acetate derivative were also effective at a daily dose of 25 mg/kg, causing an 80% and 60% survival after 30 days, respectively, whereas 8,14-diacetate and 8,14-dibutyrate were ineffective at the same dose. 8-Chloro-14-acetate (at a dose of 50 mg/kg) and nine other lankacidin C derivatives (at doses of 100 mg/kg) had scarcely any effect in prolonging the survival rate of tumor-bearing mice (table 3).

Under the same experimental conditions, L-asparaginase given at a daily dose of 250 IU/kg for 5 days was effective (80% survival on Day 30), while 6-mercaptopurine at a dose of 25 mg/kg and cyclophosphamide at a dose of 50 mg/kg had little effect in prolonging the survival time of tumor-bearing mice (20% and 0 survival on Day 30, respectively).

Of the 20 antibiotics showing activity, the 8,14-diacyl derivatives and 8-iso esters of lankacidin C, and the lankacidinol derivatives were considered to be less effective. Thus, only lankacidin C and its eight monoesters at position 8 or 14 were used for further studies on ascites L1210 leukemia.

Ascites L1210 Leukemia

In this experiment, the effects of the selected derivatives on the survival time of BDF_1 mice bearing ascites L1210 leukemia were compared at ip doses of 75 and 150 mg/kg. These dosages were based on the findings that lankacidin C was effective in prolonging the survival time of L1210-bearing mice at a daily dose of more than 150 mg/kg (33% ILS), while it

TABLE 1.—Summary of results on the antitumor activities of lankacidin C derivatives modified at the position 8 against ascites 6C3HED/OG lymphosarcoma*

Drug	Dosage (mg/kg/day ×5)	No. of mice	Survivors on Day 30 (%)	Treated/control— Mean survival time† (days)	Treated/control— Body weight change‡ (g)
Lankacidin C	20	9	67	18.3/13.3	3.3/7.0
8-Acetate	20	5	100	§/14.0	0.8/2.4
8-Propionate	20	5	100	§/12.6	2.8/6.0
8-Butyrate	20	9	89	19.0/13.8	1.4/2.7
8-Iso-butyrate	20	5	0	16.5/16.5	1.2/3.5
8-Valerate	20	5	100	§/12.5	1.0/2.0
8-Iso-valerate	20	5	0	15.5/16.5	1.2/3.5
8-Caprate	20	5	100	§/12.5	1.0/2.0
8-Palmitate	400	5	0	13.5/13.5	0.8/4.0

*6C3HED/OG lymphosarcoma cells were transplanted ip and drugs were injected ip for 5 consecutive days starting 24 hrs after tumor transplantation.
†Tumor death.
‡Body weight change at Day 5; average value of total mice tested.
§All mice alive on Day 30.

TABLE 2.—Summary of results on the antitumor activities of lankacidin C derivatives modified at the position 14 against ascites 6C3HED/OG lymphosarcoma*

Lankacidin C derivative	Dosage (mg/kg/day ×5)	No. of mice	Survivors on Day 30 (%)	Treated/control— Mean survival time† (days)	Treated/control— Body weight change‡ (g)
14-Acetate	25	4	75	21.0/14.4	-0.4/2.4
14-Propionate	25	5	80	24.0/14.4	0.4/2.4
14-Butyrate	20	4	25	14.3/14.0	2.2/3.4
14-Iso-butyrate	20	5	80	22.0/14.0	2.0/3.4
14-Valerate	20	4	0	13.2/14.0	2.8/3.4
14-Iso-valerate	20	4	25	14.6/14.0	2.8/3.4
14-Caprate	20	4	100	§/14.0	1.7/3.4
14-Palmitate	400	5	0	13.5/13.5	1.0/4.0

*6C3HED/OG lymphosarcoma cells were transplanted ip and drugs were injected ip for 5 consecutive days starting 24 hrs after tumor transplantation.
†Tumor death.
‡Body weight change at Day 5; average value of total mice tested.
§All mice alive on Day 30.

was ineffective at a daily dose of 75 mg/kg (11% ILS) (9).

The results are summarized in table 4. The lankacidins 8-butyrate, 8-propionate, and 14-caprate were effective at a daily dose of 75 mg/kg causing a 28%, 33%, and 47% ILS, respectively. 8-Caprate was the most effective in prolonging the survival time of L1210-bearing mice but was rather toxic at a dose of 150 mg/kg causing a decrease in the body weight. The lankacidins 14-butyrate (13% ILS), 14-propionate (26% ILS), and 14-valerate (29% ILS) were relatively less effective at a dose of 150 mg/kg (table 4).

Growth Inhibition of Solid 6C3HED/OG Lymphosarcoma

The effects of lankacidin C and eight of its derivatives on tumor growth were studied by measuring the size of 6C3HED/OG lymphosarcoma transplanted sc into C3H/He mice (tables 5 and 6).

When administered ip, lankacidin C inhibited tumor growth dose dependently and the minimum effective dose was found to be between 50 and 100 mg/kg (table 5). Thus, the effects of the eight derivatives of lankacidin C were evaluated at ip doses of 100 mg/kg. Under these experimental conditions,

TABLE 3.—Summary of results on the antitumor activities of 15 other lankacidin-group antibiotics against ascites 6C3HED/OG lymphosarcoma*

Drug	Dosage (mg/kg/day ×5)	No. of mice	Survivors on Day 30 (%)	Treated/control— Mean survival time† (days)	Treated/control— Body weight change‡ (g)
Lankacidin C					
8, 14-Diacetate	100	5	100	§/11.4	1.4/2.2/
8, 14-Dibutyrate	100	5	100	§/16.5	1.0/3.5/
8, 14-Disuccinate	100	5	0	16.5/18.5	2.0/2.6
8-Chloro-14-acetate	50	5	0	19.0/18.5	1.4/2.6
14-Oxo-8-propionate	100	5	0	16.7/20.7	0.2/2.2
6, 7, 12, 13-Tetrahydro	100	5	0	15.7/18.5	1.8/2.6
Lankacidinol	100	5	100	§/13.0	0.8/2.0
8-Acetate	100	5	40	14.7/13.4	1.2/3.0
14-Acetate	100	5	100	§/13.0	0.8/2.0
2′, 14-Diacetate	100	5	0	18.5/18.5	2.0/2.6
2′, 8, 14-Triacetate	100	5	40	16.0/13.4	1.4/3.0
18-ol, 14-Acetate	100	5	0	18.0/16.7	1.0/2.2
Isolankacidinol	100	5	60	15.5/14.8	1.4/2.8
Isolankacidinol O	100	5	20	14.0/14.8	1.2/2.8
Lankacyclinol	100	5	0	13.0/13.4	3.4/3.0

*6C3HED/OG lymphosarcoma cells were transplanted ip and drugs were injected ip for 5 consecutive days starting 24 hrs after tumor transplantation.

†Tumor death.

‡Body weight change at Day 5; average value of total mice tested.

§All mice alive on Day 30.

TABLE 4.—Summary of results on the antitumor activities of lankacidin-group antibiotics on the survival time of mice bearing L1210 leukemia*

Drug	Dosage (mg/kg/day × 5)	No. of mice	Treated/control— Mean survival time (days)	Treated/control— Body weight change† (g)	ILS (%)
Lankacidin C‡	75	5	9.7/8.7	2.0/3.3	11
	150	5	11.4/8.6	0/4.3	33
	250	5	12.4/8.0	0.8/1.8	55
	500	5	14.2/8.0	-1.6/1.8	78
	1000	5	18.2/8.0	-3.0/1.8	128
8-Propionate	75	15	11.2/8.4	0.9/3.7	33
	150	10	12.7/8.3	1.0/4.0	53
8-Butyrate	75	4	11.0/8.6	3.0/1.0	28
	150	4	12.5/8.6	2.5/1.0	45
8-Valerate	75	4	11.0/9.0	-1.8/3.5	22
	150	4	20.5/10.2	-1.2/2.6	101
8-Caprate	75	5	21.3/10.2	0.6/2.6	109
	150	5	19.4/10.2	-2.4/2.6	90
14-Propionate‡	75	12	10.2/8.6	2.3/2.8	19
	150	10	10.8/8.6	1.5/2.0	26
14-Butyrate	75	4	9.5/8.6	1.0/1.0	11
	150	4	9.7/8.6	-0.7/1.0	13
14-Valerate	150	5	13.2/10.2	2.0/2.6	29
14-Caprate	75	5	15.0/10.2	0.6/2.6	47
	150	5	14.0/10.2	-0.6/2.6	37
Cyclophosphamide	50	5	14.0/10.2	0.2/1.4	37

*L1210 leukemia cells were transplanted ip and drugs were administered daily ip for 5 days starting 24 hrs after tumor transplantation.

†Mean body weight change on the last day of drug injection.

‡Results of 2-3 separate experiments described previously (*from* ref 9).

TABLE 5.—Effects of ip administration of lankacidin C and its derivatives on the growth of sc transplanted 6C3HED/OG lymphosarcoma*

Drug	Dosage (mg/kg/day × 9)	No. of mice	Tumor size† (mm) (mean ± SD)	Inhibition (%)	Body weight change‡ (g)
Lankacidin C	25	5	15.7±1.4	9	2.9
	50	5	13.1±1.7	24	2.7
	100	5	5.4±1.1	69	2.5
	150	5	3.3±0.9	81	0.6
8-Propionate	100	5	4.1±1.0	76	1.6
8-Butyrate	100	5	8.8±1.7	50	1.6
8-Valerate	100	5	13.6±1.0	22	2.5
14-Propionate	100	5	13.1±1.7	25	1.4
14-Butyrate	100	5	11.9±4.1	32	1.7
14-Valerate	100	5	17.5±1.4	0	2.7
14-Caprate	100	5	16.9±1.4	2	2.1
Cyclophosphamide	25	5	8.7±1.5	50	0.5
Control		5	17.5±2.2		2.6

*Tumor cells were transplanted sc and drugs were administered ip for 9 consecutive days starting 24 hrs after tumor transplantation. 8-Caprate was not tested in this experiment.
†Tumor size was measured 11 days after tumor transplantation.
‡Mean body weight change on the last day of drug injection.

TABLE 6.—Effects of oral administration of lankacidin C and its derivatives at a dose of 1500 mg/kg on the growth of sc transplanted 6C3HED/OG lymphosarcoma*

Drug	Dosage (mg/kg/day × 8)	No. of mice	Tumor size† (mm) (mean ± SD)	Inhibition (%)	Body weight change‡ (g)
Lankacidin C	1500	5	13.6±0.8	16	2.2
8-Propionate	1500	5	14.3±0.6	12	2.1
8-Butyrate	1500	5	11.6±1.8	28	2.4
8-Valerate	1500	5	3.3±1.0	80	-1.2
8-Caprate	1500	5	10.0±4.8	38	1.2
14-Butyrate	1500	5	3.5±1.5	78	0
14-Valerate	1500	5	2.0±1.6	88	0.8
14-Caprate	1500	5	3.6±1.0	78	-2.0
Cyclophosphamide	100	5	1.1±0.8	93	2.0
Control		5	16.2±2.2		3.7

*Tumor cells were transplanted sc and drugs were administered orally for 8 consecutive days starting 24 hrs after tumor transplantation. 14-Propionate was not tested in this experiment.
†Tumor size was measured 11 days after tumor transplantation.
‡Mean body weight change on the last day of drug injection.

8-propionate showed a 76% inhibition and was the most effective of the lankacidin C derivatives tested. The lankacidins 14-butyrate and 8-butyrate showed a 32% and 50% inhibition of tumor growth, respectively, and the lankacidins 8-valerate and 14-caprate had little effect (table 5).

In contrast, lankacidin C was almost ineffective when given orally, even at the high dose of 1500 mg/kg (16% inhibition) (table 6). Under similar conditions, lankacidins 8-propionate and 8-butyrate were relatively ineffective (causing 12% and 28% inhibition, respectively), while 14-caprate, 14-butyrate, 8-valerate, and 14-valerate were highly effective (78%, 78%, 80%, and 88% inhibition, respectively) and 8-caprate was moderately effective (38% inhibition) (table 6). In addition, in another experiment 8-caprate and 14-caprate were effective at an oral dose of 1000 mg/kg (causing 32% and 67% inhibition, respectively) and 14-butyrate was the most effective in retarding tumor growth when given orally at a

daily dose of 500 mg/kg (43% inhibition). 14-Propionate was almost ineffective at a daily dose of 1000 mg/kg (6% inhibition).

Prolongation of the Survival Time of Mice Bearing Solid L1210 Leukemia

Lankacidin C has been shown to be effective in retarding the growth of sc implanted L1210 leukemia when given ip in a dose range of 300-600 mg/kg (9). In this study therefore, the efficiency of the eight derivatives of lankacidin C were compared at ip doses of 300 mg/kg starting 24 hours after tumor implantation. None of these derivatives was effective either in prolonging the survival time of tumor-bearing mice or in retarding tumor growth (table 7).

Oral administration of lankacidin C, 8-propionate, or 14-butyrate in somewhat high doses prolonged the survival time of mice bearing solid L1210 leukemia. At a dose of 1500 mg/kg, lankacidin C and 8-propionate prolonged the survival time by 23% and 27% respectively, and 14-butyrate prolonged the survival time by 44% (table 8).

TABLE 7.—Effects of ip administration of lankacidin C and its derivatives on the survival time of mice bearing solid L1210 leukemia*

Drug	Dosage (mg/kg/day × 5)	No. of mice	Mean survival time (days)	ILS (%)	Body weight change† (g)
Lankacidin C	300‡	5	11.8	26	-0.8
8-Propionate	300‡	5	11.4	21	1.2
8-Butyrate	300	5	11.2	9	2.5
8-Valerate	300	5	11.2	9	-2.6
8-Caprate	300	5	9.4	0	2.3
14-Propionate	300	5	9.6	0	1.1
14-Butyrate	300	5	9.0	0	-5.8
14-Valerate	300	5	9.6	0	-1.2
14-Caprate	300	5	10.0	0	-2.2
Cyclophosphamide	50	5	14.0	37	-0.2
Control		5	10.2		1.4
Control‡		5	9.4		1.6

*One million L1210 cells were transplanted sc and drugs were administered ip for 5 days starting 24 hrs after tumor transplantation.
†Mean body weight change at Day 5.
‡Results of a separate experiment.

TABLE 8.—Effects of oral administration of lankacidin C, 8-propionate, and 14-butyrate on the survival time of mice bearing solid L1210 leukemia*

Drug	Dosage (mg/kg/day × 5)	No. of mice	Mean survival time (days)	ILS (%)	Body weight change† (g)
Lankacidin C	1000	5	11.2	8	0.2
	1500	5	12.8	23	-0.4
8-Propionate	1000	5	11.6	12	0
	1500	5	13.2	27	-0.2
14-Butyrate	1000	5	14.2	37	1.0
	1500	5	15.0	44	-0.7
Cyclophosphamide	50	5	15.2	46	-0.4
Control		5	10.4		0.1

*One million L1210 cells were transplanted sc and drugs were administered orally for 9 days starting 24 hrs after transplantation.
†Mean body weight change on the last day of drug injection.

Suppressive Effects on Antibody Production

Previously, it was found that lankacidin C possessed relatively weak, but distinct immunosuppressive activity against anti-sheep erythrocytes antibody production at a daily dose of 300 mg/kg given for 4 days from 24 hours after immunization (9). In this work, to confirm the immunosuppressive activity of lankacidin C and to examine the effects of its derivatives on serum antibody production, somewhat high daily doses of the drugs were used. Repeated ip administration of lankacidin C at a dose of 1000 mg/kg for 4 days to C57BL/6 mice starting 24 hours after immunization of the mice with sheep erythrocytes resulted in marked reduction of hemagglutinin production on the fifth day after the antigen (fig 2). On the contrary, six derivatives of lankacidin C given at the same dose regimen were far less active in immunosuppression than lankacidin C. Among these, three derivatives modified at the position 8 of lankacidin C, 8-propionate, 8-butyrate, and 8-valerate, showed slight immunosuppressive activity, whereas the other three derivatives, modified at the position 14, 14-propionate, 14-butyrate, and 14-valerate, were completely ineffective. Under similar experimental conditions, lankacidins 8-caprate and 14-caprate were toxic.

On the other hand, repeated oral administrations of eight derivatives of lankacidin C as well as lankacidin C itself to immunized C57BL/6 mice at a dose of 1000 mg/kg for 4 days resulted in no reduction of hemagglutinin production against sheep erythrocytes (fig 3). Under the same experimental conditions, 6-mercaptopurine given at a dose of 100 mg/kg and cyclophosphamide given at a dose of 50 mg/kg suppressed antibody production significantly (fig 3).

Suppressive Activity on the GVHR

In a preliminary experiment, it was found that lankacidin C also had an inhibitory effect on the GVHR. In this experiment, the immunosuppressive activity of lankacidin C was reconfirmed by comparing it with that of lankacidin C derivatives given at the minimal effective dose of lankacidin C.

As can be seen in table 9, when lankacidin C was administered ip to BDF_1 mice at a dose of 500 mg/kg for 5 days from 24 hours after transfer of C57BL/6 spleen cells, the GVHR was significantly inhibited by 64% of controls. Similarly, the lankacidins 14-caprate, 8-caprate, and 8-valerate, given in the same dose regimen suppressed the GVHR by 63%, 65%, and 74%, respectively.

Under similar experimental conditions five other lankacidin C derivatives showed scarcely any suppressive activity. On the contrary, an enhanced GVHR was observed in the group of mice treated with 14-butyrate (table 9).

DISCUSSION

These experiments confirmed previous results showing that lankacidin C and two of its derivatives, 8-propionate and 14-propionate, have considerable antitumor activity and that they showed less immunosuppressive activity than lankacidin C (9). It was also demonstrated that 17 of 29 newly prepared lankacidin-group antibiotics, such as lankacidinol, isolankacidinol, lankacidinol 14-acetate, and 14 other derivatives of lankacidinol C, had considerable antitumor activity against ascites 6C3HED/OG lymphosarcoma (tables 1-3). Of these 17 active antibiotics, three monoester derivatives of lankacidin C modified at position 8 and 14-caprate were highly effective in prolonging the survival time of tumor-bearing mice at a daily dose of 20 mg/kg, and the others were effective at a daily dose of 100 mg/kg. However, neither of the oxidation products of the hydroxy group at position 14 nor the reduction products of the carboxyl group at position 18 and the double bonds in lankacidin C showed antitumor activity. These results suggest that the unique stereochemical structure of lankacidin C is essential for expression of antitumor activity.

Moreover, it was shown that the lankacidins 8-propionate, 8-valerate, 8-butyrate, 8-caprate, and 14-caprate were more active against ascites L1210 leukemia than lankacidin C (table 4) when given ip, but when given orally, the lankacidins 14-valerate, 14-butyrate, 14-caprate, and 8-valerate were more effective in retarding the tumor growth of solid 6C3HED/OG lymphosarcoma than lankacidin C (table 6). These results substantiate the previous suggestion that replacement of the hydroxy group at the position 8 of lankacidin C by an acyloxy group would potentiate the antitumor activity when given ip, whereas substitution at position 14 might strengthen the antitumor activity when administered orally. These differences between the two groups of derivatives may possibly be caused by differences in the absorption rates, distributions, or fates of the antibiotics in the body. However, paradoxically, the immunosuppressive activities (fig 2, table 9) and host toxicities (7) of these lankacidin C derivatives were significantly reduced irrespective of whether they were modified at position 8 or 14.

It has been found that orally administered ^{14}C-14-propionate is slowly absorbed and widely distributed in the tissues of mice. Metabolites of the antibiotic are excreted mainly in the bile in the form of lankacidin C, lankacidinol, and lankacyclinol (8). Thus, the antimicrobial activity of 14-propionate, in

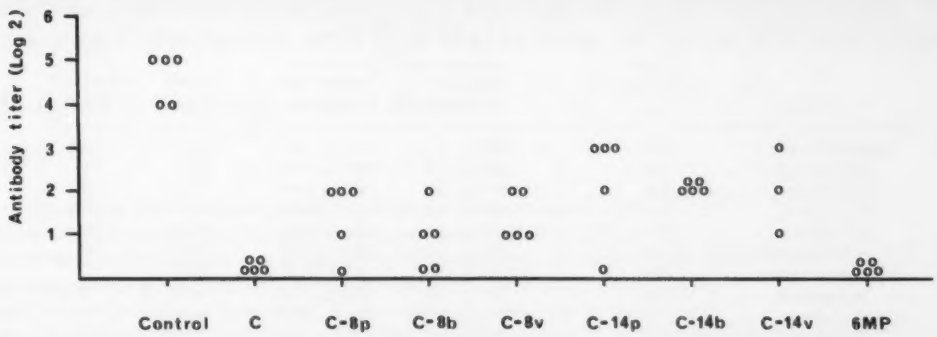

FIGURE 2.—Effects of ip administration of lankacidin-group antibiotics on antibody production in C57BL/6 mice against sheep erythrocytes. All mice were immunized by iv injection of 1×10^8 sheep erythrocytes and bled 5 days after immunization. Each point is the value for 1 animal. Drugs were injected ip for 4 consecutive days,starting 24 hours after antigen-administration. Lankacidin-group antibiotics (1000 mg/kg): C = lankacidin C; C-8p = 8-propionate; C-8b = 8-butyrate; C-8v = 8-valerate; C-14p = 14-propionate; C-14b = 14-butyrate; C-14v = 14-valerate; and 6MP = 6-mercaptopurine (100 mg/kg).

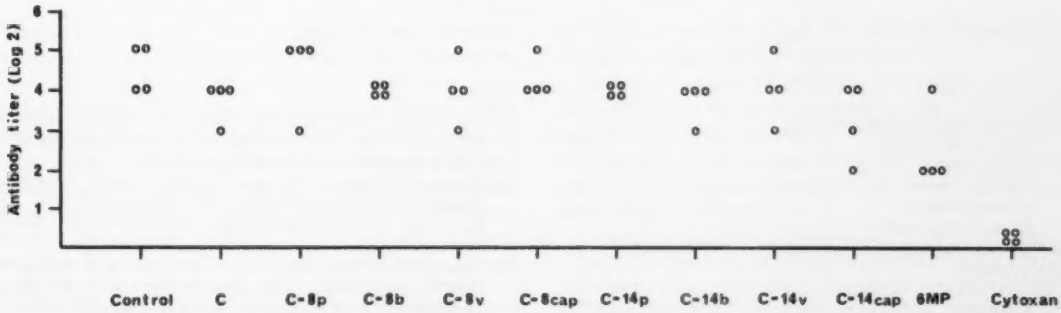

FIGURE 3.—Effects of oral administration of lankacidin-group antibiotics on antibody production in C57BL/6 mice against sheep erythrocytes. Drugs were injected as described in fig 2. Lankacidin-group antibiotics (1000 mg/kg): C= lankacidin C; C-8p = 8-propionate; C-8b = 8-butyrate; C-8v = 8-valerate; C-8cap = 8-caprate; C-14p = 14-propionate; C-14b = 14-butyrate; C-14v = 14-valerate; C-14cap = 14-caprate; 6MP = 6-mercaptopurine (100 mg/kg); Cytoxan = cyclophosphamide (50 mg/kg).

vivo, may be due mainly to the activity of lankacidin C and lankacidinol, because lankacidin C shows the strongest antimicrobial activity among the lankacidin-group antibiotics and lankacidinol shows relatively weak antimicrobial activity, whereas lankacyclinol has no antimicrobial activity (7). In this experiment, we found that lankacidinol possesses antitumor activity (table 1). Our results imply that lankacidinol per se as well as lankacidin C are likely to participate in the antitumor activity of the effective monoester derivatives of lankacidin C in vivo.

LIST OF COMPOUNDS

L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2H-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan

Lankacidin-group antibiotics:
Lankacidin C: NSC-145118; CAS reg. No. 23623-31-6
Derivatives of lankacidin C modified at position 8:
8-acetate
8-propionate
8-butyrate
8-iso-butyrate
8-valerate
8-iso-valerate
8-caprate
8-palmitate
Derivatives of lankacidin C modified at position 14:
14-acetate: NSC-145117; CAS reg. No. 23477-98-7
14-propionate
14-butyrate
14-iso-butyrate
14-valerate
14-iso-valerate
14-caprate
14-palmitate

TABLE 9.—Effects of lankacidin-group antibiotics on the GVHR in adult BDF_1 mice*

Drug	Dosage (mg/kg/day × 5 ip)	Spleen weight (mg)		GVHR index†	Inhibition† (%)
		With cell transfer	Without cell transfer		
Lankacidin C	500	66.7 ± 2.1	123.7 ± 2.3	1.85	64
8-Propionate	500	65.3 ± 3.8	189.7 ± 3.2	2.91	20
8-Butyrate	500	80.0 ± 4.3	201.3 ± 15.4	2.52	36
8-Valerate	500	85.3 ± 1.3	138.0 ± 12.5	1.62	74
8-Caprate	500	66.7 ± 5.5	123.0 ± 33.9	1.84	65
14-Propionate	500	75.3 ± 6.9	225.3 ± 17.2	2.99	16
14-Butyrate	500	74.0 ± 2.0	361.3 ± 11.8	4.88	‡
14-Valerate	500	61.0 ± 9.6	212.0 ± 14.5	3.48	0
14-Caprate	500	172.0 ± 24.2	3[illegible].2 ± 83.5	1.87	63
6-Mercaptopurine	40	99.3 ± 8.7	83.3 ± 4.6	0.84	100
Control		69.0 ± 5.1	233.2 ± 15.1	3.38	

*$(C57BL/6 \times DBA/2)F_1$ mice were injected iv with 4×10^7 C57BL/6 spleen cells, and spleen weights were measured 10 days later; drugs were injected ip for 5 consecutive days starting 24 hrs after cell transfer; groups of 3 mice were examined.

†See Materials and Methods section for explanation of GVHR index and inhibition (%).

‡GVHR was increased.

Other derivatives of lankacidin C:
- 8-chloro-14-acetate
- 8,14-diacetate
- 8,14-dibutyrate
- 8,14-disuccinate
- 14-oxo-8-propionate
- 6,7,12,13-tetrahydro

Lankacidinol

Derivatives of lankacidinol:
- 8-acetate
- 14-acetate
- 2′, 14-diacetate
- 2′, 8,14-triacetate
- 18-ol, 14-acetate

Isolankacidinol

Isolankacidinol O

Lankacyclinol

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

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Differential Sensitivity of Colony-Forming Cells of Hemopoietic Tissue, Lewis Lung Carcinoma, and B16 Melanoma to Three Nitrosoureas [1,2]

N. M. Blackett, V. D. Courtenay, and S. M. Mayer[3,4]

SUMMARY

A comparison has been made of the effects of three nitrosoureas (BCNU, CCNU, and methyl-CCNU) on the cells of Lewis lung carcinoma and B16 melanoma in vivo using a new in vitro method for assaying colony-forming cells. In addition, the effect on early hemopoietic precursors has been studied using the in vivo spleen colony assay and the in vitro agar colony assay. The results show that the three nitrosoureas are selective against Lewis lung carcinoma and B16 melanoma relative to normal hemopoietic precursors. Furthermore, the effect of these nitrosoureas is unchanged when the rate of proliferation of the hemopoietic precursor cells is increased.

[Cancer Chemother Rep 59:929-933, 1975]

The effect of single doses of three nitrosoureas on the cells of Lewis lung carcinoma and B16 melanoma that are responsible for their regrowth has been compared with the effect on the early hemopoietic precursors that contribute to the regeneration of hemopoiesis. The drugs were administered to tumor-bearing mice. Tumor-cell survival was measured by an in vitro colony assay (1) and survival of bone marrow precursors was measured by the in vivo spleen colony method of Till and McCulloch (2) and by the in vitro agar colony method of Metcalf (3).

METHODS

All three nitrosoureas (BCNU, CCNU, and methyl-CCNU) were formulated in the same way by dissolving the drug in dimethyl sulfoxide (~40 mg/ml) and then diluting these with 5% Tween 80 in saline to the required concentration for injection intraperitoneally (ip). This method is advantageous in that any precipitation of the drug that may occur will do so in the form of small very thin crystals which appear to be absorbed after injection. Earlier studies with 5% Tween 80 alone resulted in poor solubilization, especially for CCNU which was much less effective than the other two nitrosoureas. An alternative method, using Intralipid (4), was not found to be superior to the method employed.

Both the Lewis lung tumor and the B16 melanoma were grown in the gastrocnemius muscle of the hind leg, and the drug was injected when the tumors had reached a weight of approximately 0.5 g. The tumor and femoral bone marrow were removed 18 hours after drug injection.

Cell suspensions of the tumors were prepared using 0.25% trypsin in phosphate-buffered saline. The cells were then resuspended and diluted in Ham's F12 medium with 20% fetal bovine serum and 0.3% agar with the addition of washed red blood cells, to a concentration of 3×10^8 cells/ml, and heavily irradiated tumor cells to bring the total nucleated cell number to 10^4/ml. One milliliter of this mixture was plated over a 0.6-ml underlayer of 0.5% agar in 35-mm diameter Petri dishes and incubated at 37°C in an atmosphere of 5% oxygen, 5% carbon dioxide, and 90% nitrogen. The addition of red blood cells, the use of a reduced oxygen tension, and, in the case of B16 melanoma, the addition of heavily irradiated cells are essential to obtain plating efficiencies in the range of 30%-60%.

For hemopoietic tissue, the spleen colony assay (CFU) that was employed has been described by Till and McCulloch (2), and the in vitro agar colony assay (ACU) followed the method of Metcalf (3).

RESULTS

The dose-survival curves for CFU, ACU, Lewis lung carcinoma, and B16 melanoma are shown in figures 1-3 for BCNU, CCNU, and methyl-CCNU, respectively. The tumors and bone marrow cells

[1]Received Feb 20, 1975; revised May 12, 1975; accepted May 22, 1975.

[2]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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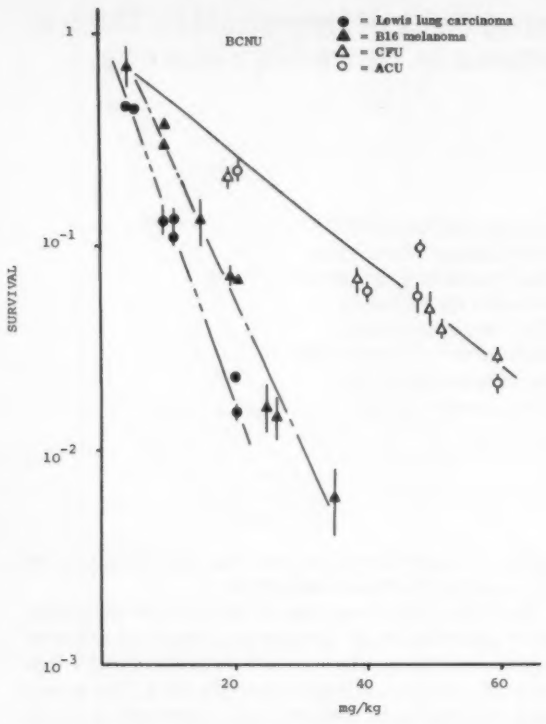

FIGURE 1.—Cell survival curves for BCNU. Vertical bars represent ± one standard error.

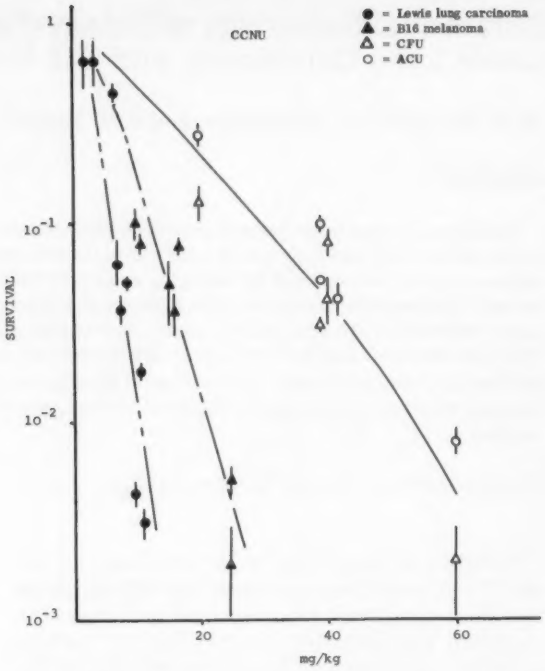

FIGURE 2.—Cell survival curves for CCNU. Vertical bars represent ± one standard error.

were removed 18 hours after the drug was injected. With all three agents, the Lewis lung tumor was more sensitive than the B16 melanoma, and both tumors were more sensitive than CFU and ACU. No difference between CFU and ACU has been observed with these three agents. For all four cell types, the toxicity, in terms of mg/kg, increased in the following drug order: BCNU, CCNU, and methyl-CCNU. The selectivity of the nitrosoureas against Lewis lung carcinoma and B16 melanoma relative to bone marrow stem cells can be seen by comparing the doses required to reduce cell survival to 1%. These values are shown in table 1 along with the ratio of the dose for CFU to those doses for Lewis lung carcinoma and B16 melanoma. In addition, data from this laboratory on cyclophosphamide (5)[5] and on γ-rays (6) have been included. Of the nitrosoureas, the smallest differential effect is for BCNU against B16 melanoma, and the greatest differential effect is for methyl-CCNU against Lewis lung carcinoma.

Cyclophosphamide has a smaller differential effect than BCNU, and tumor cells are more resistant to radiation than bone marrow stem cells; this probably would also be true if all of the tumor cells were fully oxygenated.

Preliminary studies with other cytotoxic agents such as vinblastine, methotrexate, cytosine arabinoside, and adriamycin indicate that these agents have very little effect on these two tumor types when given as single doses.

The dependence of the effect of these nitrosoureas on the rate of bone marrow cell proliferation was measured by using mice that had received 2 weeks of continuous γ-irradiation. This period of continuous irradiation has been shown in studies of thymidine suicide (7) and by repeatedly administering cytosine arabinoside (unpublished report) to cause a marked increase in the rate of stem cell proliferation. The ratio of CFU cell survival in these irradiated mice to that in normal mice was 1.26 ± 0.17 for 50 mg/kg of BCNU, 0.70 ± 0.15 for 40 mg/kg of CCNU, and 0.83 ± 0.13 for 30 mg/kg of methyl-CCNU, indicating that these agents have no appreciable proliferation dependence for bone marrow spleen colony-forming cells.

The recovery of CFU and ACU after equally effective doses of BCNU, CCNU, and methyl-CCNU (fig 4) shows that there is no difference between the three

[5]Steel GG, and Adams K. Stem cell survival and tumor control in the Lewis lung carcinoma. Cancer Res 35:1530-1635, 1975.

TABLE 1.—Approximate dose required for 1% cell survival of bone marrow stem cells, Lewis lung carcinoma cells, and B16 melanoma cells, plus the ratio of this dose for bone marrow CFU and the dose for tumor cells*

Treatment	Approximate dose (mg/kg) for—				Ratio of dose for CFU to dose for—	
	CFU	ACU	B16 melanoma	Lewis lung carcinoma	B16 melanoma	Lewis lung carcinoma
BCNU	78	78	31	22	2.5	3.5
CCNU	54	54	20	10	2.7	5.4
Methyl-CCNU	43	43	9.5	6.5	4.5	6.6
Cyclophosphamide	260	360	250	100	1.04	2.6
Irradiation						
γ-rays (rads)	430	600	1700	1700	0.25	0.25

*Data for cyclophosphamide and cobalt 60 γ-rays taken from refs 5 and 6, and footnote 5.

agents. The CFU decreased slightly from the 1st to the 2nd day while the ACU started to recover immediately. By Day 7, both cell populations had recovered to about 50% of the normal level but thereafter recovered very slowly.

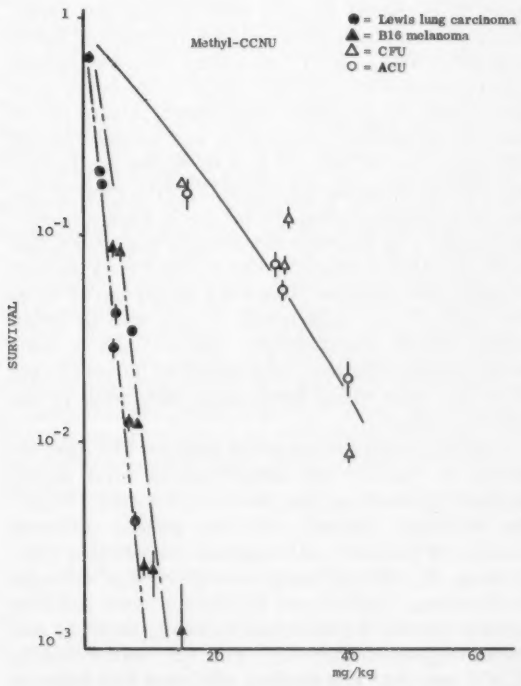

FIGURE 3.—Cell survival curves for methyl-CCNU. Vertical bars represent ± one standard error.

On Days 3 and 7 the proliferation rate was determined by measuring the effect of four injections of cytosine arabinoside (200 mg/kg/injection) over a period of 8 hours. In normal animals, cytosine arabinoside reduces the survival of CFU to about 60% (table 2), whereas 3 days after nitrosourea administration, cytosine arabinoside gives a survival rate in the range of 0.06%-0.09%. Seven days after nitrosourea administration, the effect of cytosine arabinoside was close to that seen in normal animals, except for methyl-CCNU where the effect was somewhat greater than normal.

DISCUSSION

The technique for cloning Lewis lung carcinoma and B16 melanoma cells in vitro (1) provides a rapid and reliable assay for these cells that are taken directly from the mouse. This method can therefore be

TABLE 2.—Effect of four injections of cytosine arabinoside (200 mg/kg) over an 8-hour period on the survival of CFU on Days 3 and 7 after nitrosourea administration

Drug	Survival rate	
	Day 3	Day 7
BCNU (50 mg/kg)	0.09 ± 0.02	0.67 ± 0.12
CCNU (40 mg/kg)	0.06 ± 0.02	0.58 ± 0.11
Methyl-CCNU (30 mg/kg)	0.06 ± 0.05	0.33 ± 0.06
Controls	0.61 ± 0.12	

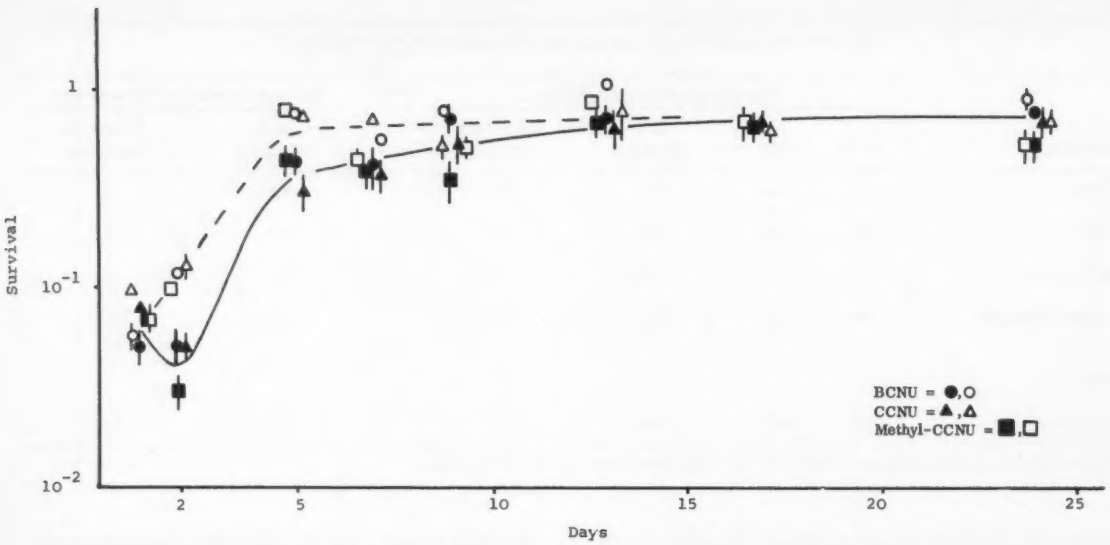

FIGURE 4.—Recovery of CFU (closed symbols) and ACU (open symbols) after single doses of BCNU (50 mg/kg), CCNU (40 mg/kg), and methyl-CCNU (30 mg/kg).

used to investigate the in vivo effects of cytotoxic agents since good agreement has been obtained between the in vivo lung colony assay and the endpoint dilution assay for both radiation (6) and cyclophosphamide.[6]

Interest in the nitrosoureas is due in part to their effectiveness against Lewis lung carcinoma and B16 melanoma since these are considered to be two of the most difficult experimental tumors to treat (8).

Our results show that each of these three nitrosoureas has a selective effect against B16 melanoma and Lewis lung carcinoma relative to two bone marrow stem cell populations: the spleen colony-forming cells and the agar colony-forming cells. The CFU measures a population of cells capable of differentiation into granulocytic, erythroid, and megakaryocytic cells while the bone marrow ACU measures cells that are already committed to granulocyte/macrophage differentiation. The Lewis lung carcinoma cells are more sensitive than the B16 melanoma cells to all three agents. With each of the four assay methods, methyl-CCNU is more effective on the basis of mg/kg of body weight than CCNU which in turn is more effective than BCNU. The greatest selectivity is shown by methyl-CCNU against Lewis lung carcinoma since for the same cell survival, the bone marrow requires about six to seven times the dose required for Lewis lung carcinoma (table 1). The least selectivity is shown by BCNU against B16 melanoma where the corresponding dose differential is only about 2.5.

This differential effect of single doses of nitrosoureas with respect to bone marrow does not necessarily explain their effectiveness in treating Lewis lung carcinoma and B16 melanoma because damage to bone marrow may not be critical to the survival of the animal. In a limited experiment, it was shown that the lethal dose in our mice was between 50 and 100 mg/kg for BCNU, 40 and 80 mg/kg for CCNU, and 30 and 60 mg/kg for methyl-CCNU; most of the deaths occurred during the 1st week. Consequently, gut seems to be the critical tissue, at least for single doses, and this is supported by the fact that the survival of bone marrow stem cells extrapolated to lethal doses is not sufficiently low to lead to hemopoietic failure. Furthermore, the recovery of bone marrow stem cells has been shown to be very rapid after the near lethal doses that were used in the present study.

A similar stem cell recovery pattern has been observed by Preisler and Henderson (9) after BCNU administration at a dose level of 20 mg/kg; the initial depletion agreed with our results although Brown and Carbone (10) required considerably higher doses. By administering a single dose of cytosine arabinoside, Preisler and Henderson were not able to show increased proliferation during recovery and indeed suggested that CFU had not been killed by BCNU, but that the seeding efficiency had been reduced; they did not, however, investigate this theory experimentally.

[6] See footnote 5.

In the present study, repeated injections of cytosine arabinoside over a period of several hours (which is a more sensitive indicator of changes in proliferation rate) showed that after the greater initial kill that was produced by 50 mg/kg of BCNU, the stem cell proliferation rate was increased at 3 days but had returned to normal by about 7 days, which was the start of the period of slow recovery.

Sensenbrenner et al (11) reported that BCNU is more effective when administered to recipients of normal bone marrow cells at short time intervals as compared to longer time intervals after transplantation, which suggests that BCNU has less effect against rapidly proliferating stem cells. However, bone marrow repopulation and drug-induced changes in the proliferative environment could explain this observation. Consequently, this result does not necessarily contradict our conclusion that the three nitrosoureas we studied are not proliferation dependent; this conclusion is based on the lack of any change in sensitivity when hemopoietic stem cells were induced to proliferate more rapidly as a result of prior whole-body irradiation.

Successful chemotherapy treatment schedules, in general, require repeated administration of one or more drugs. Single doses, however, demonstrate that there is a selective effect against Lewis lung carcinoma and B16 melanoma relative to the bone marrow. For cyclophosphamide there is no difference in sensitivity between B16 melanoma cells and bone marrow precursors, while for Lewis lung carcinoma the selective effect is low, comparable to that for BCNU in B16 melanoma. Furthermore, the absence of proliferation dependence for the three nitrosoureas indicates that protection of the marrow will not be achieved by dose fractionation unless the interval between injections is at least a few days to allow appreciable repopulation. Some protection may be afforded to the gut, however, by fractionation with shorter intervals since the shorter cell-cycle time of crypt cells could result in a more rapid recovery of this cell population. If this is the case, then damage to bone marrow could become critical to survival and the selective effect of the nitrosoureas on the two tumor types relative to bone marrow would be of importance.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

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Assay for Citrovorum Factor (NSC-3590) in the Presence of Methotrexate (NSC-740)[1,2,3]

Bipin M. Mehta and Dorris J. Hutchison[4,5,6]

SUMMARY

Treatment of certain malignancies with high-dose methotrexate/citrovorum factor rescue has recently been adopted as an effective regimen. A microbiologic assay capable of detecting citrovorum factor in the presence of massive amounts of methotrexate has been developed using a strain of *Pediococcus cerevisiae* resistant to methotrexate. The assay described in this paper is an inexpensive and rapid method of studying the distribution kinetics of citrovorum factor.

[Cancer Chemother Rep 59:935-937, 1975]

Since the report of Djerassi et al (1) on long-term remissions after the use of infrequent but regular massive treatments with methotrexate, supplemented by supportive treatment with citrovorum factor in childhood acute leukemia, high-dose methotrexate/citrovorum factor rescue regimens have been used in the treatment of many different malignant diseases (2-7). Citrovorum factor has been used to encourage the recovery of rapidly proliferating normal cells and thus reduce the toxicity that would otherwise occur. Although treatments as described above are followed effectively in cancer chemotherapy, there is a lack of data recording the distribution of these drugs in body fluids and tissues of patients. Microbiologic disc assays have been developed for a number of antitumor agents, such as adriamycin (8), cytosine arabinoside (9-11),[7] and methotrexate (12), which not only give positive proof of the presence of the active form of the drug by the inhibition of microbial growth, but also are rapid, sensitive, and inexpensive for routine use in any laboratory. However, such an assay method for citrovorum factor, an important adjuvant in modern cancer chemotherapy, has not been reported, especially for use in the presence of relatively large amounts of methotrexate.

This report describes an assay procedure for citrovorum factor which is similar to the procedures described for antitumor agents and antibiotics; however, unlike those procedures, citrovorum factor assay involves the measurement of the zone of growth, ie, the zone of exhibition, rather than the zone of inhibition. Again it determines the concentration of citrovorum factor in the presence of relatively large amounts of methotrexate.

METHODS

An assay method for citrovorum factor was described by Sauberlich and Baumann (13) using *Leuconostoc citrovorum* ATCC 8081, which was later identified as *Pediococcus cerevisiae* (14). Hutchison and Burchenal (15) isolated an amethopterin (methotrexate; A) resistant strain of *L. citrovorum* ATCC 8081 with a 2000-fold increase in resistance to methotrexate. This methotrexate-resistant strain, which we now designate as PC/A, has been maintained in our laboratory since its isolation in stabs containing 1.5% agar in the citrovorum factor assay medium (13) supplemented with 1 ng/ml of citrovorum factor (leucovorin-calcium salt, Lederle Laboratories, Division of American Cyanamid Co., Pearl River, NY) and 20 μg/ml of methotrexate by regular transfers every 3 months.

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[2]Supported in part by Public Health Service grant CA-08748 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare; grant CI-112-P from the American Cancer Society; and a grant from the Elsa U. Pardee Foundation.

[3]Citrovorum factor: CAS reg. No. 6035-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)-methyl]amino]benzoyl]-, calcium salt; leucovorin; folinic acid.

Methotrexate: CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

[4]Laboratory of Drug Resistance and Cyto-Regulation, Memorial Sloan-Kettering Cancer Center, New York, NY.

[5]We acknowledge the excellent technical assistance of Miss Ann L. Gisolfi.

[6]*Reprint requests to:* Dorris J. Hutchison, PhD, Laboratory of Drug Resistance and Cyto-Regulation, Memorial Sloan-Kettering Cancer Center, 145 Boston Post Rd, Rye, NY 10580.

[7]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; cytarabine; ara-C.

Since the determination of citrovorum factor is carried out by measuring the zone of growth rather than the zone of inhibition, the test medium (13) contained no citrovorum factor. The assay organism PC/A was grown overnight for 18 hours at 37°C in the liquid citrovorum factor assay medium (13) supplemented with citrovorum factor (1 ng/ml). This culture was washed three times with and resuspended in 0.85% saline to an optical density of 0.2 at 600 nm (approximately 4.0×10^7 cells/ml) as measured in a Bausch and Lomb "spectronic 20" spectrophotometer; 2.5 ml of the suspension was used as inoculum for a 200-ml assay medium containing 1.5% agar. Flat-bottom plates containing 10 ml each of the seeded agar medium were prepared and stored in the cold at 4°C for at least 3-4 hours prior to use for an assay. The solutions for the standard curves were made in saline (0.85%) at concentrations of 1, 5, 10, 30, and 50 ng/ml of citrovorum factor. Paper discs (Schleicher and Schull No. 740-E) of 6.35-mm diameter were used for the assay. Five discs for each concentration of citrovorum factor were saturated with 0.02 ml of the standard citrovorum factor solutions and were placed on the agar plates after drying for at least 1½ hours in the incubator at 37°C. The plates were then incubated for 18 hours at 37°C. The diameter in millimeters of the growth zone (fig 1) was measured manually. Standard curves were plotted from the averages of five zone diameter measurements. The statistical variation in citrovorum factor determination by this method was within 10.3% ± 5.8 (SD).

RESULTS AND DISCUSSION

The important feature of this assay procedure using a methotrexate-resistant strain (PC/A) is that its citrovorum factor requirement is unchanged in the presence of relatively high concentrations of methotrexate. The growth-response curve of this organism with citrovorum factor is identical to that of the wild strain *P. cerevisiae* ATCC 8081 (PC/O) (16). Citrovorum factor was determined by this method with solutions containing a mixture of citrovorum factor (as in the standard curves) and different concentrations of methotrexate. No differences were observed in the standard curve of citrovorum factor obtained in the presence of from 0 to 1 mg/ml methotrexate (fig 2). The recovery of citrovorum factor from serum, plasma, and cerebrospinal fluid was 96%-100%.

The disc assay procedure presented here has been successfully adopted in our laboratory to determine citrovorum factor concentrations in the serum and cerebrospinal fluid of cancer patients (Memorial Hospital, NY) after treatment with high-dose metho-

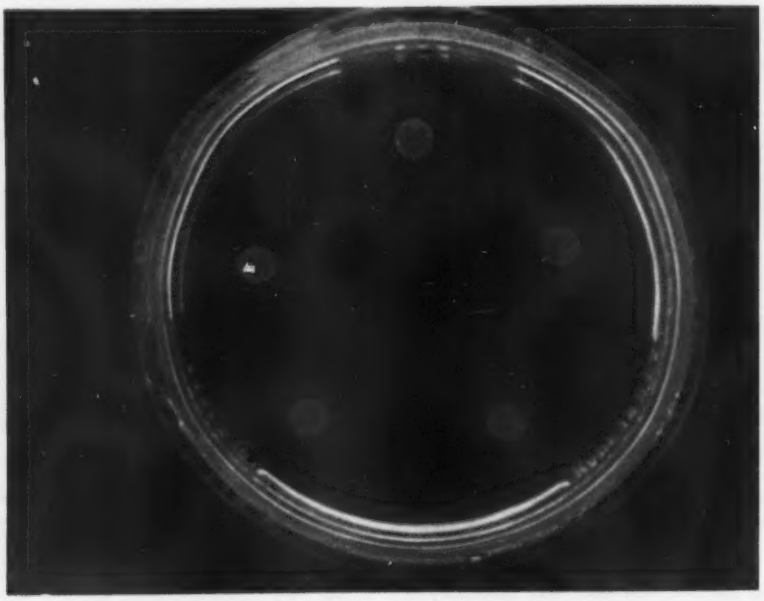

FIGURE 1.—Growth of PC/A with citrovorum factor. Zones of growth with standard solutions of citrovorum factor containing 50, 30, 10, 5, and 1 ng/ml in 0.85% saline; 0.02 ml applied to each of the respective discs (6.35-mm diameter). Assay medium (ref 13) does not contain citrovorum factor.

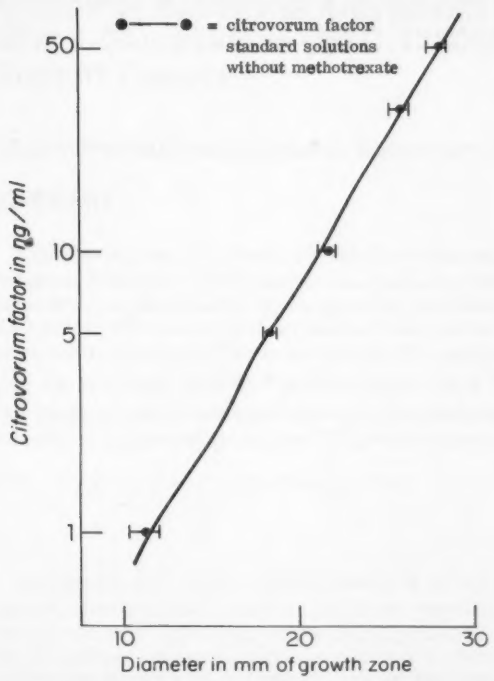

FIGURE 2.—Effect of methotrexate on citrovorum factor assay with PC/A. Prior to assay, methotrexate at 0, 10, 100, and 1000 μg/ml was added to each of the standard citrovorum factor solutions. The variations due to the presence and absence of methotrexate are represented by bars.

trexate. Children received 200-500 mg/kg of methotrexate via a 4-hour intravenous (iv) infusion and adults received 1-5 g/m² via a 24-hour iv infusion. This was followed by citrovorum factor rescue: 9-12 mg orally every 6 hours × 12; 2 hours after the methotrexate in children, and one tenth of the methotrexate dose via iv push 24 hours after the methotrexate in adults. Citrovorum factor concentrations of 1 ng/ml (limit of sensitivity, fig 2) and above could be measured in the presence of up to 1 mg/ml methotrexate, which enables one to study the distribution kinetics of citrovorum factor in the presence of methotrexate and to evaluate the effectiveness of such high-dose methotrexate/citrovorum factor rescue therapy.

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Antitumor Activity of Amygdalin MF (NSC-15780) as a Single Agent and With β-Glucosidase (NSC-128056) on a Spectrum of Transplantable Rodent Tumors[1,2,3]

Isidore Wodinsky and Joseph K. Swiniarski[4,5]

SUMMARY

Experiments are described in which four transplantable rodent tumors (L1210 lymphoid leukemia, P388 lymphocytic leukemia, B16 melanoma, and Walker 256 carcinosarcoma) were used to investigate the antitumor activity of amygdalin MF. Amygdalin MF was given alone and in combination with β-glucosidase which was administered ½ hour prior to amygdalin MF, starting 24 hours after tumor implantation. No antitumor activity was observed in any of the four tumor systems tested with the drug alone or in combined therapy. The combined therapy showed potentiation of toxicity with doses of amygdalin MF greater than or equal to 100 mg/kg.

[Cancer Chemother Rep 59:939-950, 1975]

Amygdalin MF, found in the kernels of bitter almonds, peaches, and apricots, has been reported to have been used in cancer chemotherapy since 1845 (1). In spite of its availability for 130 years, there is a striking paucity of published data on the antitumor effects of amygdalin MF on experimental tumor systems. The mechanism proposed to explain the activity of amygdalin MF is that sufficient amounts of hydrogen cyanide (HCN) are released in the presence of β-glucosidase to stop tumor respiration which is lethal for the tumor cells (fig 1) (2). HCN is reported to be less lethal for normal tissues because they contain the enzyme thiosulfate sulfurtransferase EC 2.8.1.1 (rhodanese) which converts the HCN in the presence of thiosulfate to thiocyanate (3). Burk et al (4,5) reported that Ehrlich ascites cells, treated in vitro, are sensitive to combined treatment with amygdalin MF and β-glucosidase because of the synergistic effects of the HCN-benzaldehyde mixture which produces a decreased respiration and an increased aerobic glycolysis, whereas Levi et al (6) have reported that the samples of amygdalin MF they tested on human tumor tissues and Ehrlich ascites cells had no significant effect on respiration or on aerobic and anaerobic glycolysis. Levi et al also found no significant inhibition of DNA, RNA, or protein synthesis.

The lack of in vivo experimental data prompted the DR&DP, DCT, NCI to initiate a series of experiments on a spectrum of transplantable rodent neoplasms with amygdalin MF alone and in combination with β-glucosidase at our laboratory. The results of these tests are reported herein.

MATERIALS AND METHODS

Compounds

Amygdalin MF solutions were prepared fresh daily in physiologic saline. A volume of 0.4 ml was administered intraperitoneally (ip) each day for 9 days starting 24 hours after tumor implantation.

β-Glucosidase was dissolved in cold saline each day and injected ip ½ hour prior to the amygdalin MF treatment daily for 9 days. According to the DR&DP, the β-glucosidase was obtained from Calbiochem catalog No. 346801 which contained approximately 800 IU/mg. The mice received a maximum of 160 IU/mouse/injection and the rats received a maximum of 400 IU/rat/injection.

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[2]Supported by contract NO1-CM-33727 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Amygdalin MF (NSC-B900540; lot No. 7209): CAS reg. No. 672-72-0; D-mandelonitrile, gentiobioside; Laetrile. Obtained by Drug Research and Development Program (DR&DP), DCT, NCI from the Food and Drug Administration, and prepared by the McNaughton Foundation, Montreal, Canada.

β-Glucosidase (batch MB) was obtained from Calbiochem, San Diego, Calif, by DR&DP, DCT, NCI.

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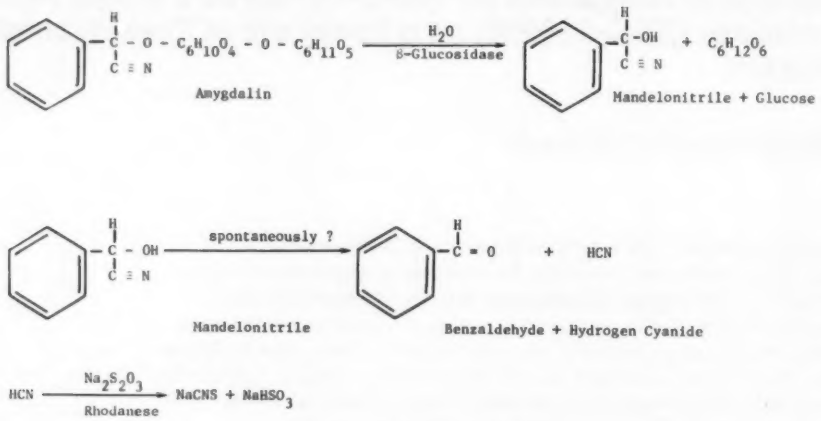

FIGURE 1.—Amygdalin metabolism.

Neoplasms

The L1210 and P388 leukemias, B16 melanoma, and Walker 256 carcinosarcoma test systems used in these studies are described elsewhere (7). These transplantable rodent-tumor systems have, in retrospective studies, detected most of the currently active clinical drugs (8).[6]

Briefly, in the L1210 leukemia test system, CDF_1 mice were inoculated ip with 10^5 tumor cells from the ascitic fluid of donor DBA/2 mice. Daily injections of amygdalin MF and β-glucosidase alone and in combination were given starting 24 hours after tumor implantation. The β-glucosidase was administered ½ hour prior to the amygdalin MF injection. The test was evaluated by determining the mean survival times of test groups of ten mice as a percentage of the mean survival time of the control group of 30 mice (T/C). It is also reported as percent increase in lifespan (%ILS) or T/C − 100. (Significant activity is ≥ 25% ILS. Significant toxicity is ≤ −15%).

In the P388 leukemia test system, CDF_1 mice were inoculated ip with 10^6 cells from the ascitic fluid of donor mice and the same procedure used in the L1210 leukemia test system was employed with the exception that an increase in median survival time was the criterion for activity.

In the B16 melanoma test system, 0.5 ml of a 1:10 g/ml tumor brei was inoculated ip or subcutaneously (sc) into BDF_1 mice; treatment began 24 hours after tumor implantation and was administered daily for 9 days. The parameter for presumptive activity is an ILS of 25%, which is considered to warrant further study.

In the Walker 256 carcinosarcoma studies, 10^6 ascites cells were implanted intramuscularly (im) in the hind leg muscle of Sprague Dawley rats and drug treatments of single 0.4-ml daily doses were given on Days 1, 3, and 6 after tumor implantation. The parameter for activity was an increase in median survival time of a treated group of ten rats, compared with the control group of 30 rats.

RESULTS

L1210 Leukemia Test System

Amygdalin MF did not increase the lifespan of CDF_1 mice bearing ip implanted L1210 leukemia when administered as a single daily dose for 9 days at dose levels of 6.25-800 mg/kg, and toxicity was not observed as evidenced by the changes on Day 5 in mean body weight and death of the mice. β-Glucosidase (10 mg/kg) was also ineffective and nontoxic. There was no increase in lifespan noted for any group treated with β-glucosidase ½ hour prior to the amygdalin MF injection. Early deaths (due to the drug) were noted in combined therapy with amygdalin MF (100 mg/kg) and β-glucosidase (10 mg/kg) (table 1).

Amygdalin MF was tested in a second experiment at dose levels from 25 to 3200 mg/kg. The results showed that the mice gained weight with amygdalin MF alone at the higher doses but no antitumor effect was noted. The mice died at the same time as the controls. β-Glucosidase was inactive and nontoxic at 10 mg/kg. Combinations of 200-800 mg/kg of amygdalin MF and 10 mg/kg of β-glucosidase were toxic; all

[6]Venditti JM. Plan for antitumor screening in animals and selection of new agents as candidates for clinical trial. *In* Report of the Division of Cancer Treatment, NCI, 1973. Bethesda, Md, NCI, 1973, vol 2, pp 2.27-2.75.

TABLE 1.—Effect of combination chemotherapy with amygdalin MF and β-glucosidase on the survival time of CDF_1 mice bearing L1210 leukemia*

Drug	Dose** mg/kg/inj.	Δ $\bar{X}$ Body Weight Grams (Day 5)	Mortality Distribution (Day) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean Survival Time (Days)
Control	0	+1.0								6	12	7	5										9.4
β-glucosidase	10	+1.5								3	4	3											9.0
Amygdalin MF	800	+2.9								1	5	4											9.3
	400	+0.9		1						3	4	3											9.0
	200	+1.0									6	2	1										9.4
	100	+0.9								3	3	3	1										9.2
	50	+1.4								1	3	5	1										9.6
	25	+0.9								4	3	3											9.2
	12.5	+0.9								4	4	1	1										8.9
	6.25	+0.7									2	6				2							10.6
β-glucosidase and Amygdalin MF	10 + 100	-				2	4			1	3												6.3
	10 + 50	+1.7								3	4	2	1										9.1
	10 + 25	+1.1								5	3	2											8.7
	10 + 12.5	+1.4								2	5	3											9.1
	10 + 6.25	+1.0								3	3	4											9.1

* L1210 - 10^5 cells in 0.1 ml i.p.
** β-glucosidase - administered i.p. on days 1-9 1xday 1/2 hr prior to amygdalin
Amygdalin - administered i.p. on days 1-9 1xday

Exp. L6381

mice died after the first injection. At 100 mg/kg of amygdalin MF and 10 mg/kg of β-glucosidase, the toxicity was evidenced by the lower increased mean lifespan (table 2).

P388 Leukemia Test System

The results were similar to the L1210 leukemia test system. In the first experiment, amygdalin MF alone or β-glucosidase alone was not effective and was nontoxic for mice bearing P388 lymphocytic leukemia. Amygdalin MF (6.25-100 mg/kg) and β-glucosidase (5 or 10 mg/kg) in combination therapy were neither active or toxic (table 3).

These same doses of β-glucosidase were employed in a second experiment but the dose levels of amygdalin MF were higher (100-400 mg/kg). The results (table 4) showed no toxicity or activity with either drug alone but when the drugs were combined, the 200- and 400-mg/kg doses of amygdalin MF were toxic with both the 5- and the 10-mg/kg dose levels of β-glucosidase. At the highest nontoxic combined treatment dose levels, no antitumor activity was noted.

Ip B16 Melanoma Test System

The results using this test system were similar to those noted with the L1210 and P388 leukemia test systems. Amygdalin MF and β-glucosidase used as single agents showed no antitumor activity, as evidenced by the parameter of increased survival time, and no toxicity, as evidenced by drug-related deaths and mean change in body weight. There is some evidence, again, that the combination of 100 mg/kg of amygdalin MF and 10 mg/kg of β-glucosidase was toxic for the BDF_1 mice. Three of the ten mice died by Day 9. The median lifespan of the group, however, was 19.5 days compared with 20 days for the control groups of mice (table 5).

Sc B16 Melanoma Test System

The results in the sc B16 melanoma assay demonstrated that both compounds were well tolerated at the highest dose level tested and, in general, the mean weight gain for the treated groups was on the positive side. Death in the control group occurred between 17 and 39 days with a median of 26.0 days, and one mouse lived past Day 45. We noted that almost all of the deaths in the treated groups fell within this range and no group survived long enough to produce a 25% ILS. There were eight mice with large ulcerated tumors alive at the end of the experiment (Day 45). Only the combination of 100 mg/kg of amygdalin MF and 10 mg/kg of β-glucosidase was toxic for the mice (table 6).

Im Walker 256 Carcinosarcoma Test System

The median survival time for a group of 30 control rats was 14.0 days with a mean weight gain of 18 g on Day 5. There were two of 30 survivors on Day 45 in which the tumor had regressed completely by Day 18. Regressions with the im implanted Walker 256 carcinosarcoma have been observed previously (9,-10).

Table 7 shows that neither amygdalin MF nor β-glucosidase administered as single agents at dose levels up to 1000 and 10 mg/kg, respectively, increased the median survival time of the tumor-bearing rats. The drugs, given in combination, were toxic for the rats at both the 250-mg/kg amygdalin MF and the 5- and 10-mg/kg β-glucosidase dose levels. The maximal ILS (21%) was noted in this experiment at a combined dose of 31.3 mg/kg of amygdalin MF and 10 mg/kg of β-glucosidase.

Because of this observation, a second experiment was initiated (table 8) using an overlapping range of amygdalin MF (1.95-62.5 mg/kg) with β-glucosidase (1.25-10 mg/kg) administered as single agents and in combination. The results of this experiment showed no antitumor activity and no toxicity for the tumor-bearing rats with all combinations or with the drugs alone.

CONCLUSION

The pioneer study by Skipper et al (11) demonstrated a positive correlation between the drug kill of leukemic cells and an increase in the survival time of the tumor-bearing hosts. The results of our experiments showed that the neoplastic cells of L1210 lymphoid leukemia, P388 lymphocytic leukemia, ip and sc implanted B16 melanoma, and im implanted Walker 256 carcinosarcoma were not sensitive to amygdalin MF or to β-glucosidase when administered as single agents or in combination. There was no significant increase in the mean or the median lifespan of tumor-bearing groups of animals at the highest nontoxic tolerated dose levels. If a significant percentage of the hosts' tumor cells had been killed by the treatment, an increase in survival time would have been expected; however, this was not observed.

Amygdalin MF was not toxic for tumor-bearing mice when injected ip at a level of 64 mg/mouse/day for 9 days, and β-glucosidase could be administered safely at a dose level of 0.2 mg/mouse. Toxicity was noted only when β-glucosidase was administered

TABLE 2.—Effect of combination chemotherapy with amygdalin MF and β-glucosidase on the survival time of CDF_1 mice bearing L1210 leukemia*

Drug	Dose** mg/kg/inj.	Δ $\bar{X}$ Body Weight Grams (Day 5)	Mortality Distribution (Day) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean Survival Time (Days)
Control	0	+1.1								1	16	12	1										9.4
β-glucosidase	10	+0.9								4	6												8.6
Amygdalin MF	3200	+1.4			1					2	2	4	1										8.8
	1600	+0.7								1	6	3											9.2
	800	+0.9								3	3	3	1										9.2
	400	+1.2								2	3	5											9.3
	200	+1.2								2	7	1											8.9
	100	+1.9								4	3	2	1										9.0
	50	+1.0								4	3	2	1										9.0
	25	+0.8								1	5	4											9.3
β-glucosidase	10 + 800	-		10																			2.0
and	10 + 400	-		10																			2.0
Amygdalin MF	10 + 200	-		10																			2.0
	10 + 100	+1.2			1					7	2												7.7
	10 + 50	+0.8								4	2	4											9.0
	10 + 25	+1.4								6	2	2											8.6

* L1210 - 10^5 cells in 0.1 ml i.p.
** β-glucosidase - administered i.p. on days 1-9 1xday 1/2 hr prior to amygdalin
Amygdalin - administered i.p. on days 1-9 1xday

Exp. L6397

TABLE 3.—Effect of combination chemotherapy with amygdalin MF and β-glucosidase on the survival time of CDF, mice bearing P388 lymphocytic leukemia*

Drug	Dose** mg/kg/inj.	Δ $\bar{X}$ Body Weight Grams (Day 5)	Mortality Distribution (Day) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Median Survival Time (Days)
Control	0	+0.5										3	15	10	1	1							11.0
β-glucosidase	10	0.0											5	3	2								11.5
	5	-0.3											4	5	1								12.0
Amygdalin MF	800	-0.1				1						1	6	2									11.0
	400	-0.9											6	4									11.0
	200	-0.2										2	3	4	1								11.5
	100	-0.2									1		3	3	3								12.0
	50	-1.5										3	3	3	1								11.0
	25	-1.4									1	1	3	4		1							11.5
	12.5	-0.8									1		6	3									11.0
	6.25	+0.5										1	3	3	2	1							12.0
β-glucosidase	10 + 100	-0.7		1								1	2	3	2	1							12.0
and	10 + 50	-0.6											6	3	1								11.0
Amygdalin	10 + 25	0.0											5	5									11.5
	10 + 12.5	-0.7											5	4	1								11.5
	10 + 6.25	-1.0									2		2	6									12.0
	5 + 100	-1.0										1	6	2									11.0
	5 + 50	-0.6									1		3	4	2								12.0
	5 + 25	-0.7									1	2	2	5									11.5
	5 + 12.5	-0.2									2		4	4									11.0
	5 + 6.25	-0.5									3		3	3	1								11.0

* P388 - 10^6 cells in 0.1 ml i.p.
** β-glucosidase - administered i.p. on days 1-9 1xday 1/2 hr prior to amygdalin
amygdalin - administered i.p. on days 1-9 1xday

Exp. PS1974

TABLE 4.—Effect of combination chemotherapy with amygdalin MF and β-glucosidase on the survival time of CDF_1 mice bearing P388 lymphocytic leukemia*

Drug	Dose** mg/kg/inj.	Δ $\bar{X}$ Body Weight Grams (Day 5)	Mortality Distribution (Day) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Median Survival Time (Days)
Control	0	+1.7										2	9	15	3	1							12.0
β-glucosidase	10	+1.2									1	3	3	3									11.0
	5	+1.6										3		5	1	1							11.0
Amygdalin MF	400	+0.9											3	6	1								12.0
	200	+0.7									1	2	5	1	1								11.0
	100	+1.2										3	5	2									11.0
β-glucosidase	10 + 400	-		10																			2.0
and	10 + 200	-		10																			2.0
Amygdalin	10 + 100	+1.1									2	4	3	1									10.0
	5 + 400	-		8	1	1																	2.0
	5 + 200	-		6		1						2	1										2.0
	5 + 100	+1.5									1	6	2	1									10.0

* P388 - 10^6 cells in 0.1 ml i.p.
** β-glucosidase - administered i.p. on days 1-9 1xday 1/2 hr prior to amygdalin
Amygdalin - administered i.p. on days 1-9 1xday

Exp. PS2052

TABLE 5.—Effect of combination chemotherapy with amygdalin MF and β-glucosidase on the survival time of BDF_1 mice bearing ip implanted B16 melanoma *

Drug	Dose** mg/kg/inj.	$\bar{\Delta}$ X Body Weight Grams (Day 5)	Mortality Distribution (Day) 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Median Survival Times (Days)
Control	0	+1.8										1	1	4	7	7	9		1								20
β-glucosidase	10	+2.3											1	4			4	1									19.5
	5	-0.4													1	2	7										21.0
Amygdalin MF	800	+2.6											1	1	2	2	2	1	1								20.0
	400	+1.9												1	2	4		2				1					20.0
	200	+2.2												1	4	3	2										19.5
	100	+2.4												1	1	2	4	2									21.0
	50	+2.1												1	3	1	3	1	1								20.5
	25	+2.1												2	2	3	3										20.0
	12.5	+1.8											1	4	1		1	1	1	1							18.5
	6.25	-0.9										1			2	3	2	1	1								20.0
β-glucosidase and Amygdalin	10 + 100	+1.6	1	2								1			1	2	1	1	1								19.5
	10 + 50	+2.0											2	1	3	1	1	2									19.0
	10 + 25	+1.8											3	2	4		1										18.5
	10 + 12.5	+0.7										1	1	3	3		1		1								18.5
	10 + 6.25	+1.0										1	1	1	2	3	1	1									20.5
	5 + 100	+1.2												2	1	1	1	4									21.5
	5 + 50	+0.9											1	2		1	6										21.0
	5 + 25	+1.8											1	2	2	1	4										19.5
	5 + 12.5	+1.7										2	2	2	2		2										18.0
	5 + 6.25	+2.8											1		4		5										20.0

* B16 melanoma - 0.5 ml of 1:10 (gm/ml) brei implanted i.p.
** β-glucosidase - administered i.p. on days 1-9 lxday 1/2 hr prior to amygdalin
Amygdalin - administered i.p. on days 1-9 lxday

Exp. B258

TABLE 6.—Effect of combination chemotherapy with amygdalin MF and β-glucosidase on the survival time of BDF_1 mice bearing sc implanted B16 melanoma *

Drug	Dose** mg/kg/inj.	Δ X̄ Body Weight Grams (Day 5)	Mortality Distribution (Day) 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45	Median Survival Time (Days)	Survivors (Day 45)
Control	0	+1.4	1 2 2 2 3 1 1 2 1 3 2 2 3 1 1 1 1 1	26.0	1/30
β-glucosidase	10	+1.2	2 2 1 1 2 1 1 2 1	24.5	1/10
	5	+1.7	1 1 1 1 1 1 1 1 1 1	25.5	1/10
Amygdalin MF	800	+1.3	1 2 3 2 1 1	24.0	0/10
	400	+1.4	1 1 1 2 3 2	26.5	0/10
	200	+1.9	1 2 1 2 2 1 1 2	25.0	0/10
	100	+0.9	1 2 2 2 1 2	22.5	0/10
	50	+1.2	1 1 1 1 1 1 1 1 1 1 1	27.0	1/10
	25	+0.8	1 1 1 3 1 1 1 1	24.0	0/10
	12.5	+1.6	2 3 2 1 2 2	22.0	0/10
	6.25	+1.3	1 1 1 1 1 1 1 3	24.5	0/10
β-glucosidase and Amygdalin MF	10 + 100	+1.0	1 3 3 1 1	9.0	1/10
	10 + 50	+0.7	3 1 1 1 1 1 1 1	25.5	0/10
	10 + 25	+0.7	1 2 1 2 1 1 1 1 1	23.0	1/10
	10 + 12.5	+0.9	1 1 1 1 2 1 1 1	25.0	1/10
	10 + 6.25	+1.2	1 2 1 2 1 1 1 1 1 1	27.0	0/10
	5 + 100	+1.8	1 1 1 1 2 1 1 2	26.0	1/10
	5 + 50	+0.9	1 1 1 1 1 1 2 2 1 1	26.5	0/10
	5 + 25	+1.3	1 1 1 2 1 1 2 1 1	26.5	0/10
	5 + 12.5	+1.0	1 1 1 2 1 1 1 1	25.0	1/10
	5 + 6.25	+1.6	1 2 1 4 1 1	29.0	0/10

* B16 Melanoma - 0.5 ml of 1:10 (gm/ml) brei implanted subcutaneously
** β-glucosidase - administered i.p. on days 1-9 1xday 1/2 hr. prior to amygdalin
Amygdalin - administered i.p. on days 1-9 1xday

Exp. B003

TABLE 7.—Influence of combination chemotherapy with amygdalin MF and β-glucosidase on the survival time of Sprague Dawley rats implanted im with Walker 256 carcinosarcoma *

Drug	Dose** mg/kg/inj.	$\bar{\Delta}$ X Body Weight Grams (Day 5)	Mortality Distribution (Day) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45	Median Survival Time (Days)	Survivors (Day 45)
Control	0	+18	4 2 3 2 6 4 2 1 1 1	14.0	2/30
β-glucosidase	10	+18	2 1 1 2 1 1 1	12.0	1/10
	5	+20	3 1 2 2 1 1	14.0	0/10
Amygdalin MF	1000	+20	1 1 3 1 1 1 1	13.5	1/10
	500	+19	1 1 4 1 3	12.0	0/10
	250	+19 1	1 1 2 3 1 1	12.5	0/10
	125	+18	1 3 1 2 2 1	13.5	0/10
	62.5	+20	2 2 1 3 1 1	12.5	0/10
	31.3	+18	1 1 3 2 1 1 1	12.5	0/10
β-glucosidase	10 + 500	-	8 2	2.0	0/10
and	10 + 250	-	8 2	2.0	0/10
Amygdalin MF	10 + 125	+15	1 1 1 3 2 1	14.0	0/10
	10 + 62.5	+13	1 1 2 1 2 1 1 1	12.5	0/10
Amygdalin MF	10 + 31.3	+16	2 1 1 2 1 1 1	17.0	1/10
	5 + 500	-	9 1	2.0	0/10
	5 + 250	-	5 2 1 1 1	3.0	0/10
	5 + 125	+14	2 2 1 2 1 2	11.5	0/10
	5 + 62.5	+18	1 1 1 1 1 2 1 1 1	14.0	0/10
	5 + 31.3	+16	1 2 2 1 1 1 1 1	15.5	0/10

* Walker 256 - 10^6 cells in 0.1 ml intramuscularly
** β-glucosidase - administered i.p. on days 1, 3 and 6 1xday 1/2 hr prior to amygdalin
Amygdalin - administered i.p. on days 1, 3 and 6 1xday

Exp. WA610

TABLE 8.—Influence of combination chemotherapy with amygdalin MF and β-glucosidase on the survival time of Sprague Dawley rats bearing im implanted Walker 256 carcinosarcoma*

Drug	Dose** mg/kg/inj.	Δ $\bar{X}$ Body Weight Grams (Day 5)	Mortality Distribution (Day) 8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Median Survival Time (Days)	Survivors (Day 45)
Control	0	+14	1		6	9	3	3	4	2												11.0	0/30
β-glucosidase	10	+16			5	3	1			1												10.5	0/10
	5	+16			4	4		1		1												11.0	0/10
	2.5	+17			2	6	1	1														11.0	0/10
	1.25	+15			5	2	1	1				1										10.5	0/10
Amygdalin MF	62.5	+17			4	3	1	1														11.0	1/10
	31.3	+16			4	1		2		1		1									1	12.0	0/10
	15.6	+15		1	4	4	1															10.5	0/10
	7.8	+15			5	4	1															10.5	0/10
	3.9	+15			3	3	2	1								1						11.0	0/10
	1.95	+17			2	3	1	1				1										11.5	2/10
β-glucosidase and Amygdalin MF	10 + 62.5	+15			6	4																10.0	0/10
	10 + 31.3	+15			6	2	1		1													10.0	0/10
	10 + 15.6	+10			1	5	2	1		1												11.0	0/10
	10 + 7.8	+15		1	2	5	1	1														11.0	0/10
	10 + 3.9	+13			4	2	1	1		2												11.0	0/10
	10 + 1.95	+16			3	5	1	1														11.0	0/10
	5 + 62.5	+14			5	1	3			1												10.5	0/10
	5 + 31.3	+17			2	4	1	1		1					1							11.0	0/10
	5 + 15.6	+18			1	5		2	2													11.0	0/10
	5 + 7.8	+17		1	3	3	2	1														11.0	0/10
	5 + 3.9	+18			3	7																11.0	0/10
	5 + 1.95	+15		1	2	2	4	1														11.5	0/10
	2.5 + 62.5	+15			6	3	1															10.0	0/10
	2.5 + 31.3	+16			1	5	1	2	1													11.0	0/10
	2.5 + 15.6	+17			3	4	1	1			1											11.0	0/10
	2.5 + 7.8	+21			4	2	2	2														11.0	0/10
	2.5 + 3.9	+16		1	3	3	1				2											11.0	0/10
	2.5 + 1.95	+16		1	2	3	1	1		1												11.0	1/10
	1.25 + 62.5	+15			4	2	1			1			1									11.0	1/10
	1.25 + 31.3	+15			5	1	2	2														10.5	0/10
	1.25 + 15.6	+15			3	4	2					1										11.0	0/10
	1.25 + 7.8	+16		1	6	2					1											10.0	0/10
	1.25 + 3.9	+17			5	3	1					1										10.5	0/10
	1.25 + 1.95	-3			7	1	1	1														10.0	0/10

* Walker 256 - 10^6 cells in 0.1 ml intramuscularly

** β-glucosidase - administered i.p. on days 1, 3, and 6 1xday 1/2 hr prior to amygdalin
Amygdalin - administered i.p. on days 1, 3, and 6 1xday

Exp. WA611

prior to doses greater than or equal to 100 mg/kg of amygdalin MF. Thus, it would appear that no differential tumor-cell/normal-cell sensitivity existed in these tumor systems and hence no successful chemotherapy resulted.

ADDENDUM

Since this paper was submitted, Hill et al have reported on the lack of therapeutic effect of amygdalin in B16 melanoma and BW5147 leukemia (12).

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Experimental Studies of the Antitumor Activity of Amygdalin MF (NSC-15780) Alone and in Combination With β-Glucosidase (NSC-128056)[1,2,3]

W. R. Laster, Jr. and F. M. Schabel, Jr.[4,5]

SUMMARY

Amygdalin MF was evaluated alone and in combination with an activating agent, β-glucosidase, against three transplantable rodent tumors; Ridgway osteogenic sarcoma, Lewis lung carcinoma, and P388 leukemia. In dose-response studies up to the LD20 in normal mice, amygdalin MF alone did not demonstrate significant antitumor activity against any of these three tumor systems. Similarly, at doses not exceeding the LD10 in normal mice, amygdalin MF plus β-glucosidase did not demonstrate antitumor activity against any of these three tumor systems. Potentiation of the lethal toxicity of amygdalin MF by β-glucosidase was observed in all studies where the two agents were given in simultaneous combination.

[Cancer Chemother Rep 59:951-965, 1975]

One of the objectives of the National Cancer Program is to discover and develop new anticancer drugs. During recent years, numerous reports have appeared in both the lay press and the news sections of scientific journals reporting the undocumented anticancer activity of amygdalin MF in man. To our knowledge, the only report of possible activity of this drug against cancer in experimental animals appeared in an undocumented "News and Comment" report in *Science*, in which it was stated that "the results clearly show that amygdalin significantly inhibits the appearance of lung metastases in mice bearing spontaneous mammary tumors and significantly increases the inhibition of the growth of the primary tumors over the appearance of inhibition in untreated animals" (1).

The DCT, NCI recognizes the need to establish the validity of any material considered to have antineoplastic properties against any tumor system, in animal or man. In an attempt to establish the anticancer properties of amygdalin MF, the DCT requested Southern Research Institute to evaluate this compound against a variety of animal tumor systems. This report will describe the results of this study.

MATERIALS AND METHODS

The material to be evaluated was identified as amygdalin MF and was considered to be a clinical sample. The analytic data on amygdalin MF, as supplied by Dr. Harry Wood (DCT), are as follows: DL-mandelonitrile-β-D-glucosido-6-β-D-glucoside, also known as isomygdalin (DL on the nitrile position).

87.4%	isoamygdalin
6.9%	water
5.7%	isopropanol
100%	

Krebs and Bouziane[6] have proposed that nitrilosides (Laetriles) exert their anticancer activity by the release of nascent hydrogen cyanide (HCN) from nitrilosides (Laetriles) by enzymatic hydrolysis with β-glucuronidase or β-glucosidase. They further propose that normal cells are not destroyed by the released HCN because the enzyme rhodanese, present in normal cells but not in tumor cells, detoxifies HCN

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[2]Supported by contract NO1-CM-43756 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Amygdalin MF (NSC-B900540; lot No. 7209): CAS reg. No. 672-72-0; D-mandelonitrile, gentiobioside; Laetrile. Obtained by Drug Research and Development Program (DR&DP), DCT, NCI, from the Food and Drug Administration, and prepared by the McNaughton Foundation, Montreal, Canada.

β-Glucosidase was obtained from Calbiochem, San Diego, Calif, by DR&DP, DCT, NCI.

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[6]Krebs ET, Jr, and Bouziane NR. Nitrilosides (Laetriles). Their rationale and clinical utilization in human cancer. *In* The Laetriles-Nitrilosides in the Prevention and Control of Cancer. Montreal, Canada, the McNaughton Foundation.

to harmless thiocynates (2). Based on these observations and interpretations, we tested amygdalin MF against three murine tumor systems, either alone or in combination with β-glucosidase. The enzyme, β-glucosidase, is known to catalyze the hydrolysis of amygdalin to yield glucose, benzaldehyde, and HCN (2). β-Glucosidase was given simultaneously with amygdalin MF in order to insure hydrolysis, which produces HCN. The activity of this lot of β-glucosidase was 1010 IU/mg (activity is expressed in international units per milligram and signifies the number of micromoles of substrate converted per minute at 30°C by 1 mg of enzyme preparation).

An extensive survey of the world medical literature on nitrilosides (Laetriles) by Krebs and Bouziane[6] has considered the theoretical, experimental, and clinical data. Based on this report, the decision was made to evaluate amygdalin MF against three transplantable rodent tumors: two solid tumors and one leukemia.

The Ridgway osteogenic sarcoma (ROS) was selected as one of the two rodent solid tumors for study. The ROS is a uniformly fatal solid tumor that is markedly sensitive to representatives of the major chemical and biologically active classes of anticancer drugs used in effective, but usually noncurative, treatment of a wide variety of malignant neoplasms in man, including many solid tumors.

Established tumors (approximately 500 mg) regress under treatment with actinomycin D, adriamycin, daunorubicin, bleomycin, cyclophosphamide, melphalan, 5-fluorouracil, arabinosylcytosine, 6-mercaptopurine, vincristine, and *cis*-dichlorodiammineplatinum(II) (3). This group includes many of the anticancer drugs of greatest current clinical usefulness. Because of this wide range of drug sensitivity, the ROS was considered to be the ideal solid tumor in experimental animals to detect the antitumor activity of amygdalin MF.

The other rodent solid tumor selected for this study was the Lewis lung carcinoma. This uniformly fatal, metastasizing solid tumor was selected because it is resistant to most agents useful in man, except the alkylating agents such as cyclophosphamide and the nitrosoureas. Thus, should amygdalin MF show antitumor properties against this resistant tumor, its potential value in human oncology would be enhanced.

The third rodent tumor selected to evaluate the antitumor properties of amygdalin MF was a methylcholanthrene-induced leukemia of DBA/2 mice, the P388 leukemia. This tumor has marked utility as a model animal leukemia system because of its sensitivity to a variety of anticancer agents in use in man today. The P388 leukemia is reproducibly sensitive to (*a*) alkylating agents (BCNU, CCNU, methyl-CCNU, melphalan, cyclophosphamide), (*b*) compounds that bind to or intercalate with DNA (actinomycin D, adriamycin, bleomycin), and (*c*) vincristine, a mitotic inhibitor. In addition, the P388 leukemia is the tumor system presently being used by the DCT to screen for natural product anticancer agents.

Ridgway Osteogenic Sarcoma (ROS)

In the study designed to determine the antitumor activity of amygdalin MF against ROS, AKD_2F_1 mice (AKR × DBA/2) were implanted subcutaneously (sc) with ROS tumor fragments weighing approximately 100 mg (± 20 mg). Treatment with amygdalin MF alone and in combination with β-glucosidase was started 24 hours after tumor-cell implant. This was done to begin treatment when the tumor-cell burden was lowest, to assure a maximum likelihood of detecting any antitumor activity of the agent or the combination.

Doses of 500, 335, and 220 mg/kg of amygdalin MF alone were used. In addition, doses of 120, 80, 53, 35, and 23 mg/kg of amygdalin MF were given in simultaneous combination with 10 mg/kg/dose of β-glucosidase. All treatments (single-agent and combination therapy) were given by the intraperitoneal (ip) route using ten mice per dose.

In addition to the tumor-bearing mice, normal (nontumor-bearing) AKD_2F_1 mice of the same sex and source were treated with the same doses of amygdalin MF alone and in combination with β-glucosidase. These mice were observed for lethal toxicity (drug toxicity controls).

The mice were identified individually, were housed in stainless steel cages, and were given Wayne Lab Blox (Allied Mills, Inc.) and water ad libitum. The mice were observed daily for deaths, and two-dimensional tumor measurements were made every 3rd or 4th day from the time of first tumor appearance until termination of the experiment. Tumor measurements were converted to weight using the formula:

$$w = \frac{a \times b^2}{2},$$

where a = length in mm, b = width in mm, and w = weight in mg.

Lewis Lung Carcinoma

Amygdalin MF alone and in combination with β-glucosidase was evaluated against both the sc and the intravenously (iv) implanted Lewis lung carcinoma. In the sc group, BDF_1 mice (C57Bl/6 × DBA/2) were implanted with 40-mg tumor fragments. In the

iv group, BDF_1 mice were given 10^6 counted tumor cells via the tail vein. The same schedule and doses used in the ROS group were used in the sc and iv Lewis lung carcinoma evaluation. Normal (nontumor-bearing) BDF_1 mice were treated with the same doses of amygdalin MF alone and in combination with β-glucosidase. These mice were observed for lethal toxicity and mean body weight changes (drug toxicity controls).

P388 Leukemia

BDF_1 mice were implanted ip with either 10^6 or 10^4 P388 leukemia cells. The same schedule and doses used in the ROS and Lewis lung carcinoma groups were used in this study. Since the strain, sex, and source of BDF_1 mice, and the day of treatment and compound preparation were identical to the Lewis lung carcinoma group, the drug toxicity controls in the Lewis lung carcinoma group were used for the P388 leukemia chemotherapy trials.

RESULTS

The results of the evaluation of amygdalin MF alone and in combination with β-glucosidase against ROS are shown in table 1 and in figures 1-3. As seen in table 1 (the days of death and the weight change data), amygdalin MF alone was less toxic than when given with the activating agent, β-glucosidase. When given alone, amygdalin MF produced 10% deaths at a dose of 500 mg/kg, 30% deaths at 335 mg/kg, and no deaths at 220 mg/kg given daily for 9 days. However, when β-glucosidase was given at 10 mg/kg/dose in combination with amygdalin MF, the highest level of amygdalin MF that could be given without exceeding the LD10 in normal mice was 53 mg/kg/dose.

Cumulative mortality plots of the ROS tumor-bearing mice treated with amygdalin MF and amygdalin MF plus β-glucosidase are shown in figure 1.

Individual tumor measurements in the untreated control and treated groups are shown in figures 2 and 3. As can be seen, no significant increase in lifespan (ILS) was observed in any of the treated groups based on median survival time. In the untreated control group, the median lifespan was 40.0 days with a range of deaths from 24 to 45 days. At doses equal to or less than the LD10 in normal mice, the maximum ILS was +10% with a range of deaths from 31 to 50 days.

Examination of the individual tumor measurements (figs 2 and 3) reveals essentially no tumor inhibition at any nonlethally toxic doses.

These data indicate that amygdalin MF alone and in combination with the activating agent, β-glucosidase (at doses ≤ LD10 in normal mice), was not significantly active in inhibiting the growth of ROS in AKD_2F_1 mice.

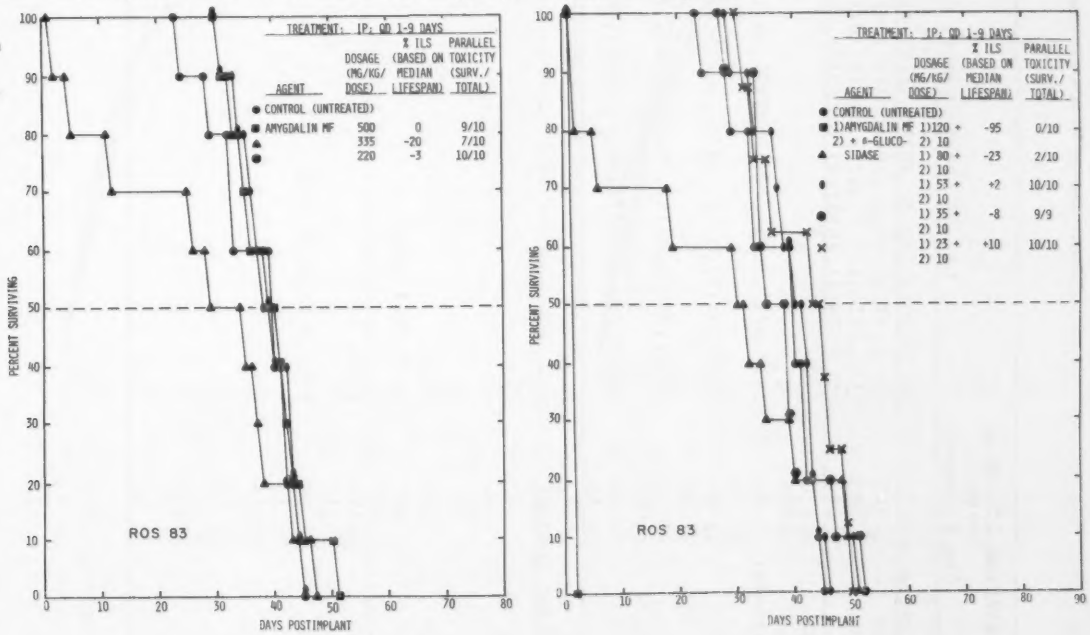

TREATMENT: IP; QD 1-9 DAYS
AGENT
DOSAGE (MG/KG/ DOSE)
% ILS (BASED ON MEDIAN LIFESPAN)
PARALLEL TOXICITY (SURV./ TOTAL)
CONTROL (UNTREATED)
AMYGDALIN MF 500 0 9/10
335 -20 7/10
220 -3 10/10
ROS 83
PERCENT SURVIVING
DAYS POSTIMPLANT
TREATMENT: IP; QD 1-9 DAYS
AGENT
DOSAGE (MG/KG/ DOSE)
% ILS (BASED ON MEDIAN LIFESPAN)
PARALLEL TOXICITY (SURV./ TOTAL)
CONTROL (UNTREATED)
1) AMYGDALIN MF 1) 120 + 2) 10 -95 0/10
2) + β-GLUCOSIDASE 1) 80 + 2) 10 -23 2/10
1) 53 + 2) 10 +2 10/10
1) 35 + 2) 10 -8 9/9
1) 23 + 2) 10 +10 10/10
ROS 83
PERCENT SURVIVING
DAYS POSTIMPLANT

FIGURE 1.—Cumulative mortality plots of ROS treated with amygdalin MF and amygdalin MF plus β-glucosidase.

TABLE 1.—Therapy for ROS with amygdalin MF alone and in combination with β-glucosidase

Treatment: IP; qd 1-9 days Name & NSC No.	Dosage (mg/kg/dose)	Day of Death (Number of Deaths)	55th-Day Survivors/ Total	Median Life Span (days)	% ILS
Control (untreated)		24(1),29(1),33(2),40(2),42(2),44(1),45(1)	0/10	40.0	
B-900540	500	31(1),33(1),35(1),36(1),39(1),41(1),42(1),43(1),45(1),51(1)	0/10	40.0	0(*)
(Amygdalin	335	2(1),5(1),12(1),26(1),29(1),35(1),37(1),38(1),43(1),47(1)	0/10	32.0	-20
MF)	220	31(1),34(1),36(1),37(1),38(1),40(1),43(2),44(1),45(1)	0/10	39.0	-3
1)B-900540	1)120	2(9)	0/9	2.0	-95
2)128056	2) 10				
(β-Glucosidase)	1) 80	2(2),6(1),19(1),30(1),32(1),35(1),40(1),49(1),51(1)	0/10	31.0	-23
	2) 10				
	1) 53	29(1),33(1),37(1),38(1),40(1),42(1),43(2),44(1),46(1)	0/10	41.0	+2(*)
	2) 10				
	1) 35	28(1),34(3),35(1),39(2),40(1),47(1),52(1)	0/10	37.0	-8
	2) 10				
	1) 23	31(1),33(1),36(1),43(1),45(1),46(1),49(1),50(1)	0/8	44.0	+10
	2) 10				

Drug Toxicity Control				Maximum Wt. Loss (gms)	Day Occurred
B-900540	500	9(1)	9/10	0	-
	335	6(1),7(1),36(1)	7/10	-1	12
	220		10/10	-1	5
1)B-900540	1)120	2(10)	0/10	-	-
2)128056	2) 10				
	1) 80	2(2),3(2),5(2),7(2)	2/10	-3	5
	2) 10				
	1) 53		10/10	-2	5
	2) 10				
	1) 35		9/9	-2	5
	2) 10				
	1) 23		10/10	-2	5
	2) 10				

*Highest nontoxic dose.

Experiment No. ROS 83.

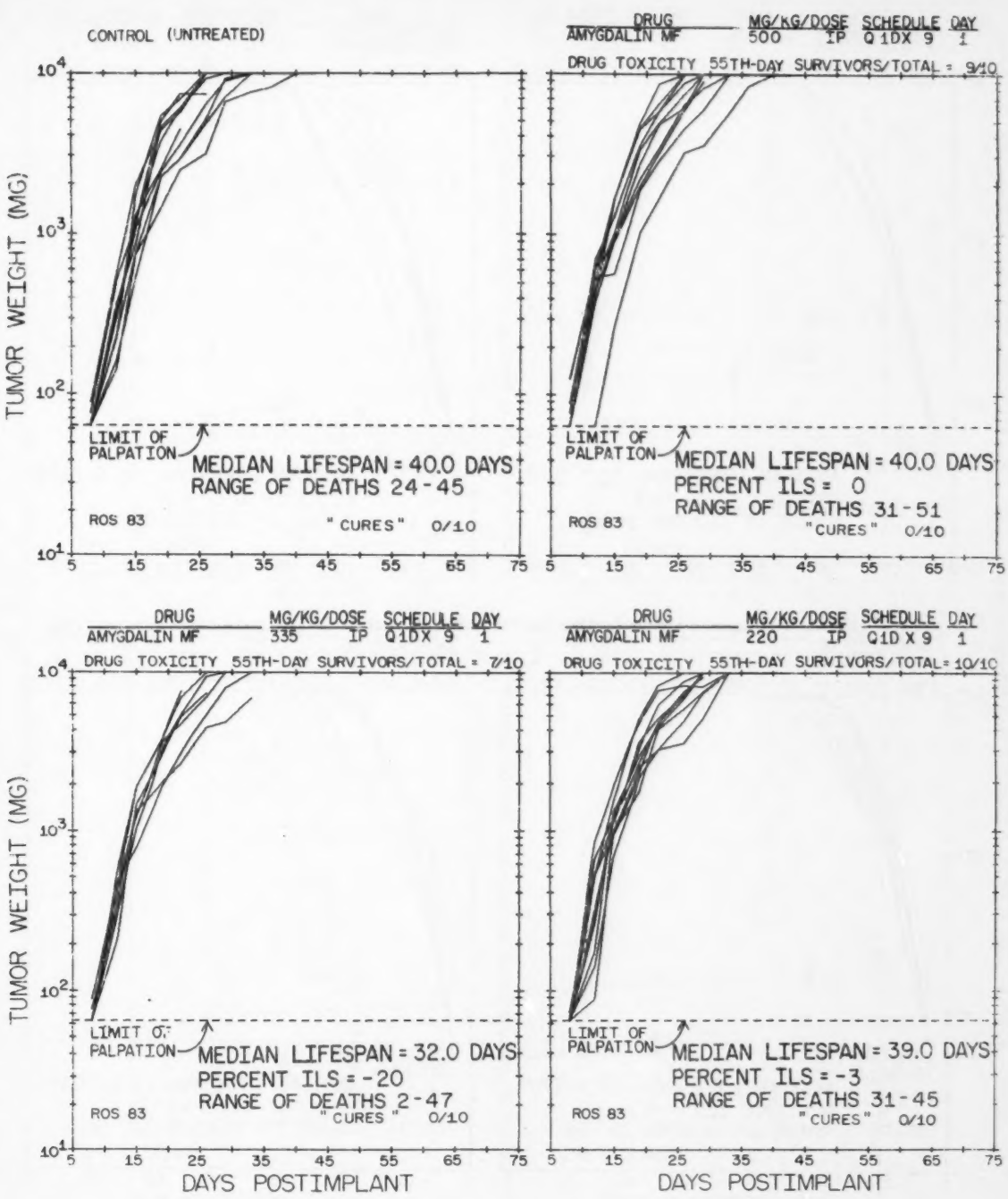

CONTROL (UNTREATED)
DRUG MG/KG/DOSE SCHEDULE DAY
AMYGDALIN MF 500 IP Q1DX 9 1
DRUG TOXICITY 55TH-DAY SURVIVORS/TOTAL = 9/10
TUMOR WEIGHT (MG)
LIMIT OF PALPATION
MEDIAN LIFESPAN = 40.0 DAYS
RANGE OF DEATHS 24 - 45
ROS 83
"CURES" 0/10
MEDIAN LIFESPAN = 40.0 DAYS
PERCENT ILS = 0
RANGE OF DEATHS 31 - 51
DRUG MG/KG/DOSE SCHEDULE DAY
AMYGDALIN MF 335 IP Q1DX 9 1
DRUG TOXICITY 55TH-DAY SURVIVORS/TOTAL = 7/10
MEDIAN LIFESPAN = 32.0 DAYS
PERCENT ILS = -20
RANGE OF DEATHS 2 - 47
DRUG MG/KG/DOSE SCHEDULE DAY
AMYGDALIN MF 220 IP Q1D X 9 1
DRUG TOXICITY 55TH-DAY SURVIVORS/TOTAL = 10/10
MEDIAN LIFESPAN = 39.0 DAYS
PERCENT ILS = -3
RANGE OF DEATHS 31 - 45
DAYS POSTIMPLANT

FIGURE 2.—Individual ROS tumor measurements in AKD_2F_1 mice treated with amygdalin MF alone.

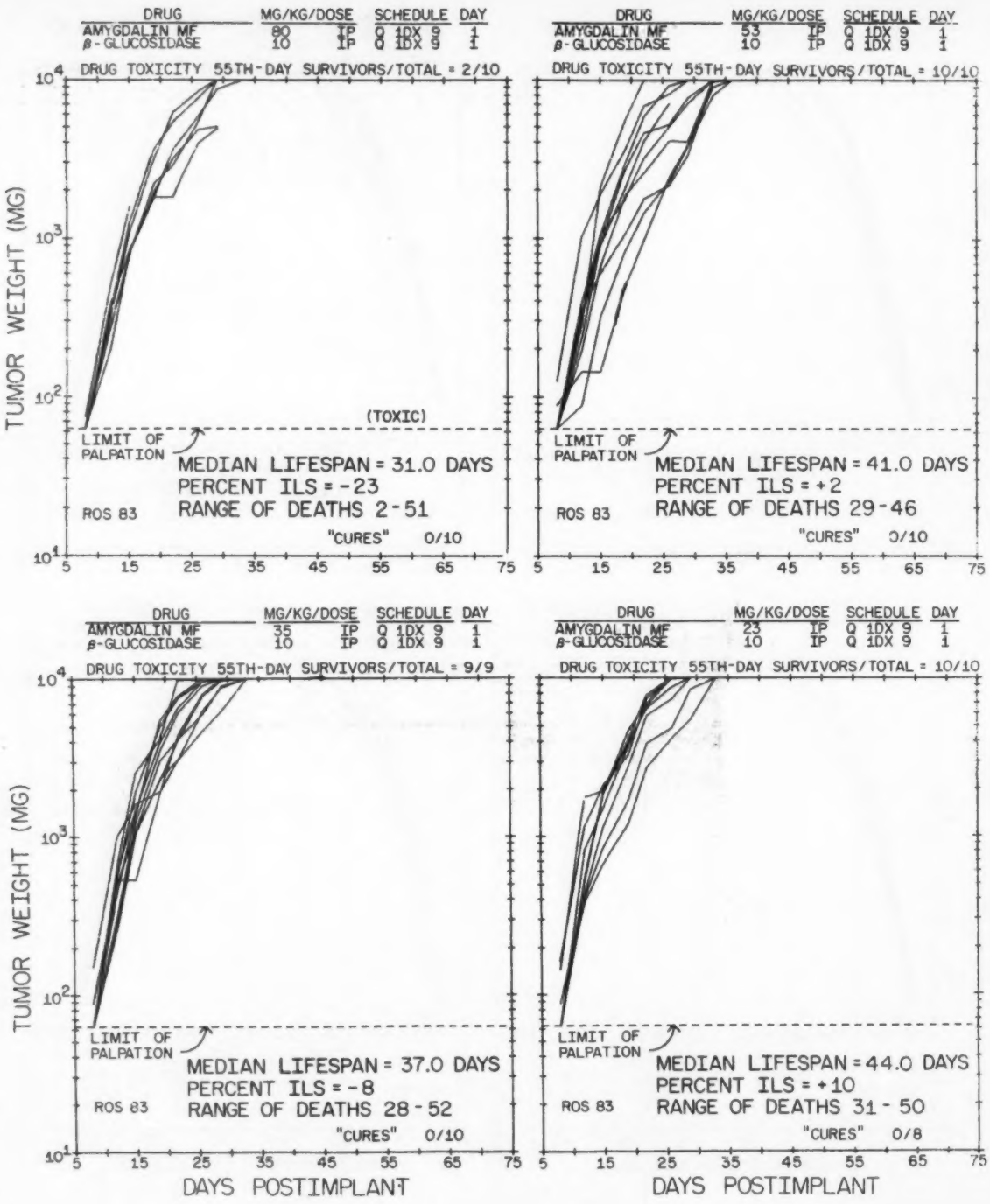

DRUG | MG/KG/DOSE | SCHEDULE | DAY
AMYGDALIN MF 80 IP Q 1DX 9 1
β-GLUCOSIDASE 10 IP Q 1DX 9 1
DRUG TOXICITY 55TH-DAY SURVIVORS/TOTAL = 2/10
(TOXIC)
LIMIT OF PALPATION
MEDIAN LIFESPAN = 31.0 DAYS
PERCENT ILS = -23
RANGE OF DEATHS 2-51
ROS 83
"CURES" 0/10
DRUG | MG/KG/DOSE | SCHEDULE | DAY
AMYGDALIN MF 53 IP Q 1DX 9 1
β-GLUCOSIDASE 10 IP Q 1DX 9 1
DRUG TOXICITY 55TH-DAY SURVIVORS/TOTAL = 10/10
LIMIT OF PALPATION
MEDIAN LIFESPAN = 41.0 DAYS
PERCENT ILS = +2
RANGE OF DEATHS 29-46
ROS 83
"CURES" 0/10
DRUG | MG/KG/DOSE | SCHEDULE | DAY
AMYGDALIN MF 35 IP Q 1DX 9 1
β-GLUCOSIDASE 10 IP Q 1DX 9 1
DRUG TOXICITY 55TH-DAY SURVIVORS/TOTAL = 9/9
LIMIT OF PALPATION
MEDIAN LIFESPAN = 37.0 DAYS
PERCENT ILS = -8
RANGE OF DEATHS 28-52
ROS 83
"CURES" 0/10
DRUG | MG/KG/DOSE | SCHEDULE | DAY
AMYGDALIN MF 23 IP Q 1DX 9 1
β-GLUCOSIDASE 10 IP Q 1DX 9 1
DRUG TOXICITY 55TH-DAY SURVIVORS/TOTAL = 10/10
LIMIT OF PALPATION
MEDIAN LIFESPAN = 44.0 DAYS
PERCENT ILS = +10
RANGE OF DEATHS 31-50
ROS 83
"CURES" 0/8
TUMOR WEIGHT (MG)
DAYS POSTIMPLANT

FIGURE 3.—Individual ROS tumor measurements in AKD_2F_1 mice treated with amygdalin MF plus β-glucosidase.

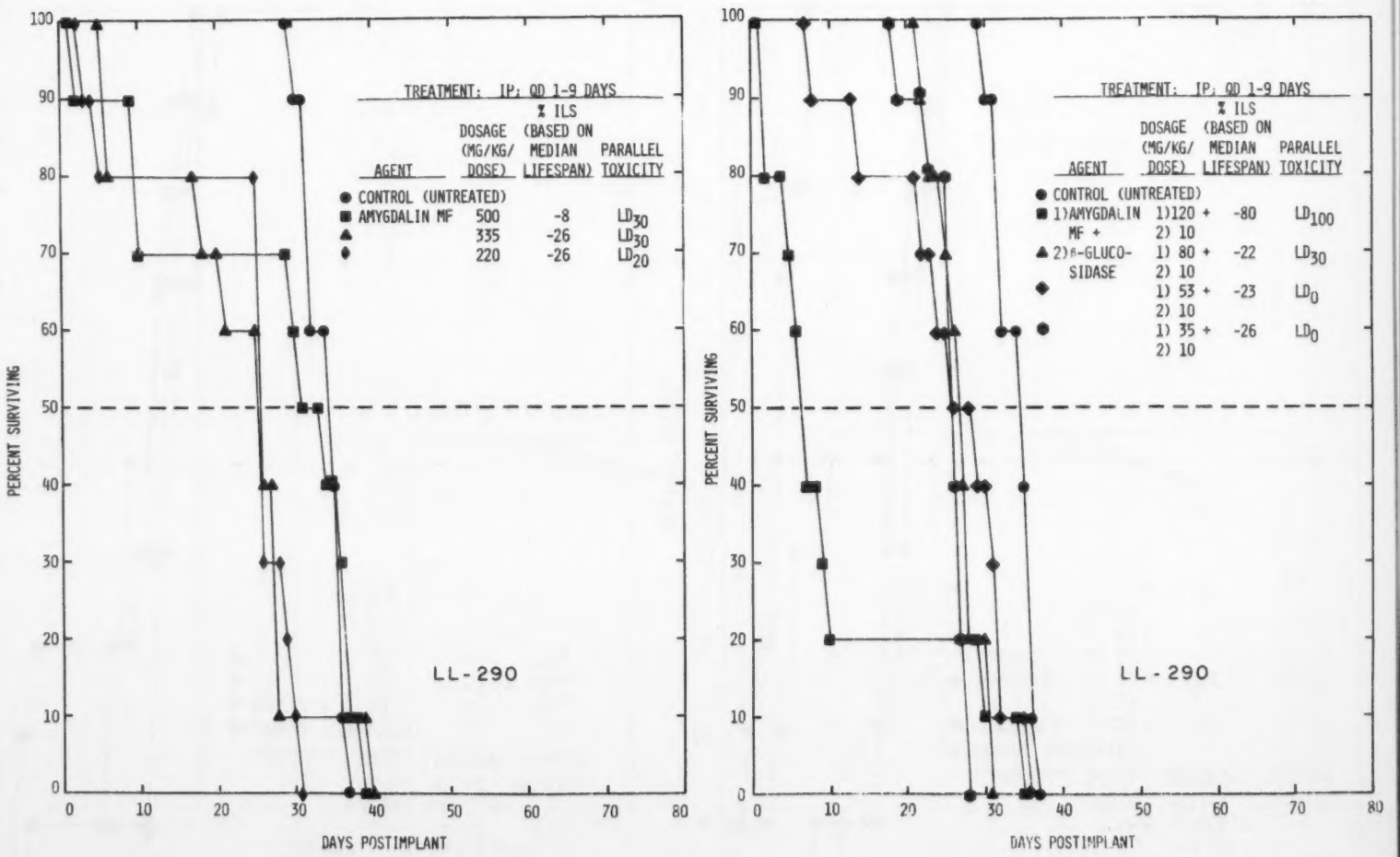
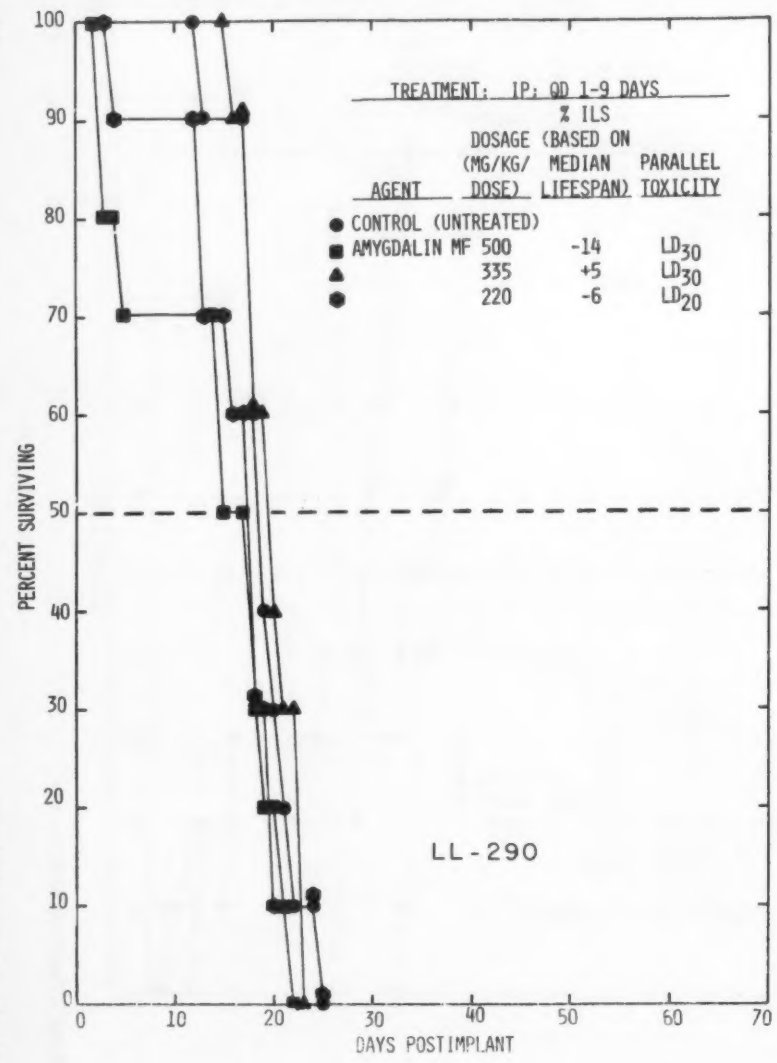


FIGURE 4.—Cumulative mortality plots of sc implanted Lewis lung carcinoma treated with amygdalin MF and amygdalin MF plus β-glucosidase.



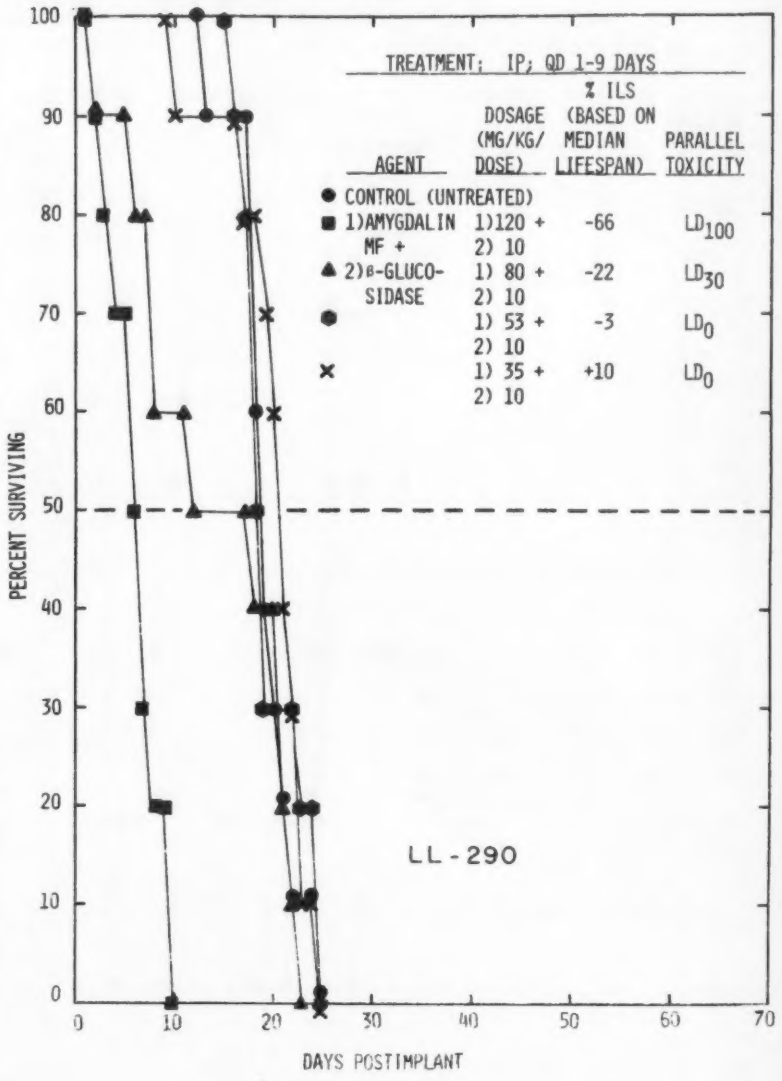


FIGURE 5.—Cumulative mortality plots of iv implanted Lewis lung carcinoma treated with amygdalin MF and amygdalin MF plus β-glucosidase.

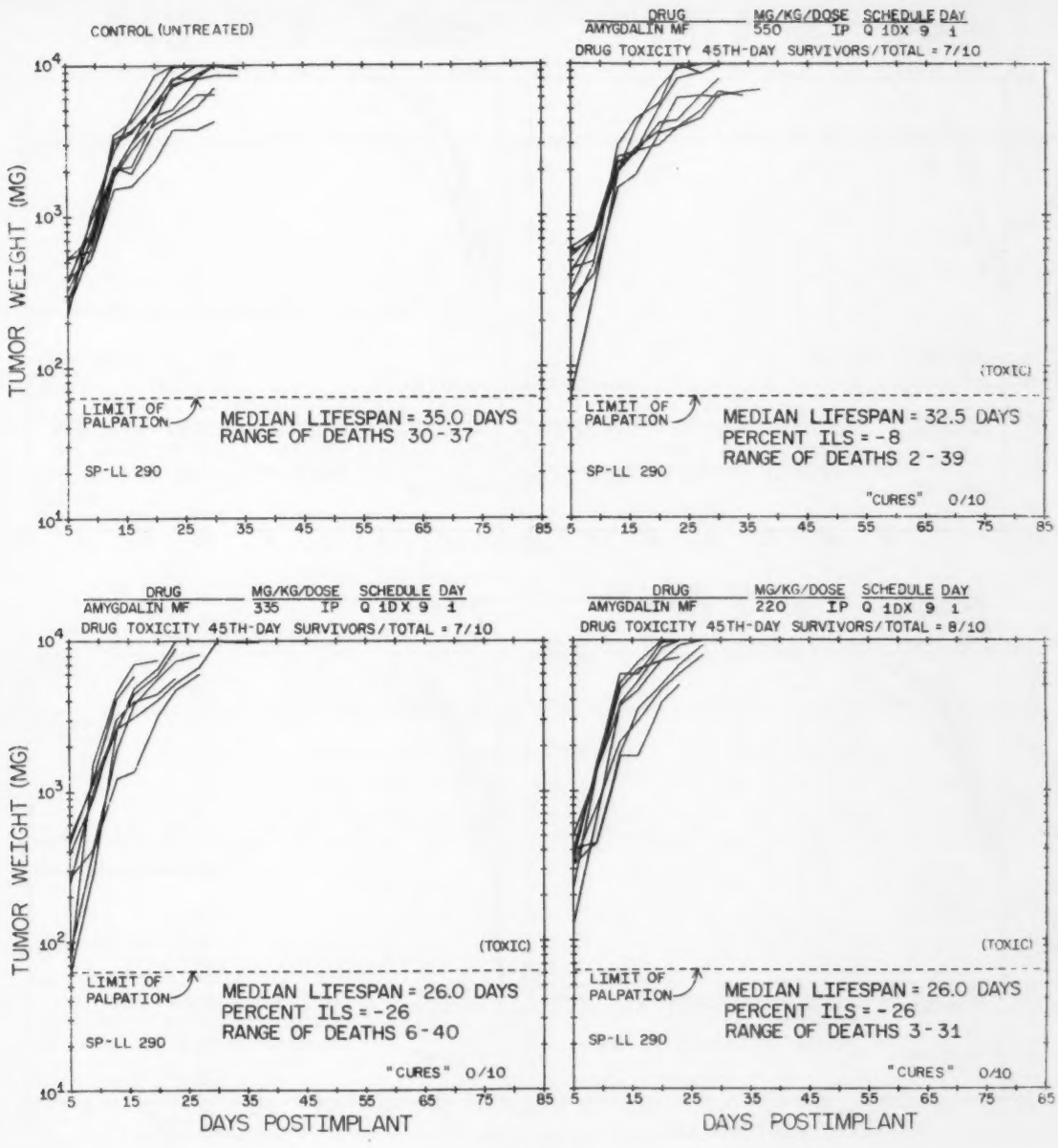


FIGURE 6.—Individual Lewis lung carcinoma measurements in BDF_1 mice treated with amygdalin MF alone.

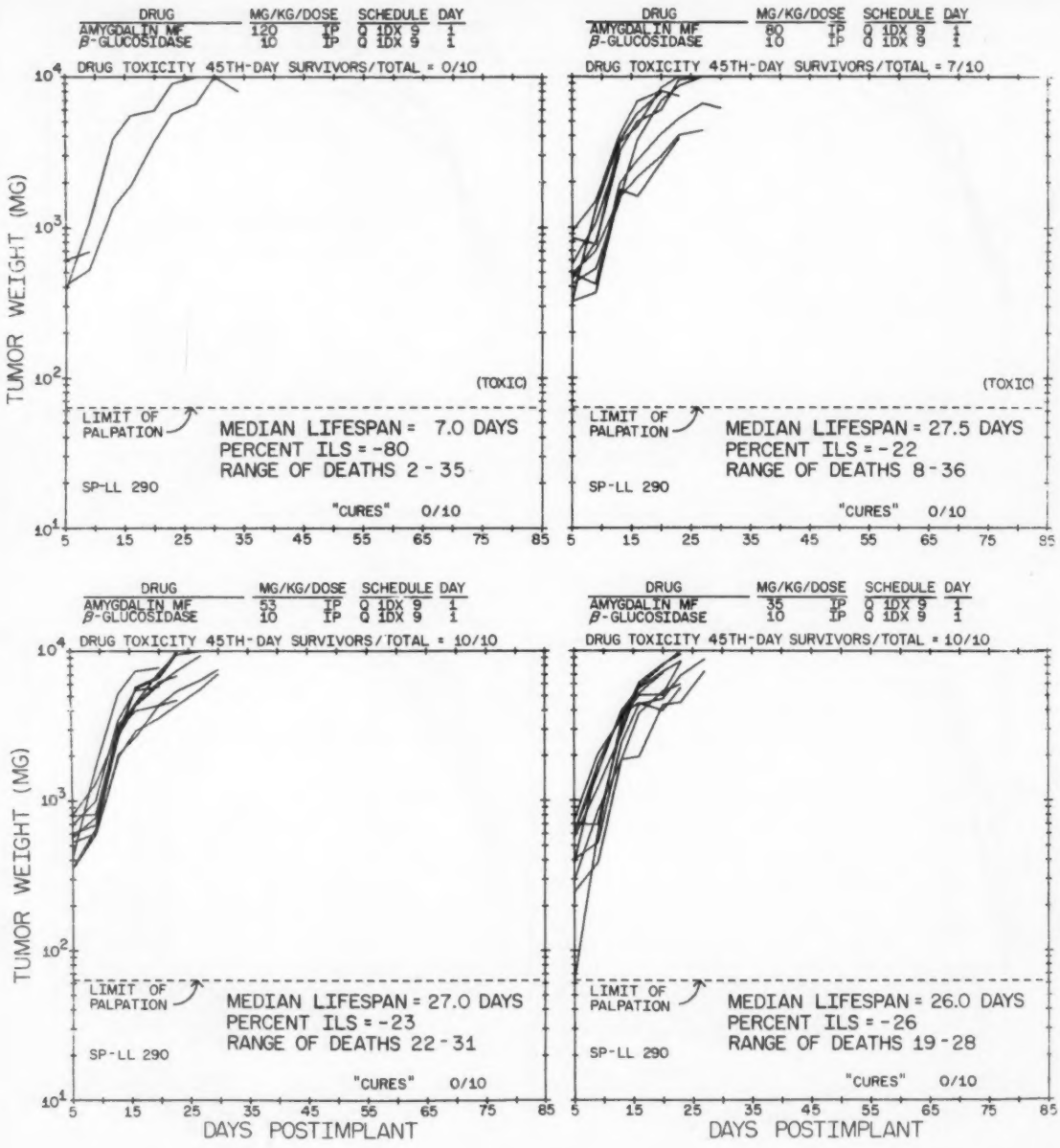

DRUG | MG/KG/DOSE | SCHEDULE | DAY
AMYGDALIN MF | 120 | IP | Q 1DX 9 | 1
β-GLUCOSIDASE | 10 | IP | Q 1DX 9 | 1
DRUG TOXICITY 45TH-DAY SURVIVORS/TOTAL = 0/10
(TOXIC)
LIMIT OF PALPATION
MEDIAN LIFESPAN = 7.0 DAYS
PERCENT ILS = -80
RANGE OF DEATHS 2 - 35
SP-LL 290
"CURES" 0/10
DRUG | MG/KG/DOSE | SCHEDULE | DAY
AMYGDALIN MF | 80 | IP | Q 1DX 9 | 1
β-GLUCOSIDASE | 10 | IP | Q 1DX 9 | 1
DRUG TOXICITY 45TH-DAY SURVIVORS/TOTAL = 7/10
(TOXIC)
LIMIT OF PALPATION
MEDIAN LIFESPAN = 27.5 DAYS
PERCENT ILS = -22
RANGE OF DEATHS 8 - 36
SP-LL 290
"CURES" 0/10
DRUG | MG/KG/DOSE | SCHEDULE | DAY
AMYGDALIN MF | 53 | IP | Q 1DX 9 | 1
β-GLUCOSIDASE | 10 | IP | Q 1DX 9 | 1
DRUG TOXICITY 45TH-DAY SURVIVORS/TOTAL = 10/10
LIMIT OF PALPATION
MEDIAN LIFESPAN = 27.0 DAYS
PERCENT ILS = -23
RANGE OF DEATHS 22 - 31
SP-LL 290
"CURES" 0/10
DRUG | MG/KG/DOSE | SCHEDULE | DAY
AMYGDALIN MF | 35 | IP | Q 1DX 9 | 1
β-GLUCOSIDASE | 10 | IP | Q 1DX 9 | 1
DRUG TOXICITY 45TH-DAY SURVIVORS/TOTAL = 10/10
LIMIT OF PALPATION
MEDIAN LIFESPAN = 26.0 DAYS
PERCENT ILS = -26
RANGE OF DEATHS 19 - 28
SP-LL 290
"CURES" 0/10
TUMOR WEIGHT (MG)
DAYS POSTIMPLANT

FIGURE 7.—Individual Lewis lung carcinoma measurements in BDF_1 mice treated with amygdalin MF plus β-glucosidase.

TABLE 2.—Evaluation of amygdalin MF and amygdalin MF plus β,glucosidase against sc and iv implanted Lewis lung carcinoma in BDF_1 mice

Name & NSC No.	Dosage (mg/kg/dose)	Day of Deaths (Number of Deaths)	45th-Day Survivors/ Total	Median Life Span (days)	% ILS
Implant: SC Fragments of Lewis Lung Carcinoma					
Treatment:IP;qd 1-9 days					
Control (untreated)		30(1),32(3),35(2),36(3),37(1)	0/10	35.0	
B-900540	500	2(1),10(2),30(1),31(1),34(1),36(1),37(2),39(1)	0/10	32.5	-8
(Amygdalin	335	6(2),18(1),21(1),26(2),28(3),40(1)	0/10	26.0	-26
MF)	220	3(1),5(1),26(5),29(1),30(1),31(1)	0/10	26.0	-26(>*)
1)B-900540	1)120	2(2),5(1),6(1),7(2),9(1),10(1),30(1),35(1)	0/10	7.0	-80
2)128056	2) 10				
(β-Glucosidase)	1) 80	8(1),14(1),22(1),24(1),26(1),29(1),31(1),32(2),36(1)	0/10	27.5	-22
	2) 10				
	1) 53	22(1),23(1),25(1),26(1),27(2),28(2),31(2)	0/10	27.0	-23(*)
	2) 10				
	1) 35	19(1),23(1),26(4),27(2),28(2)	0/10	26.0	-26
	2) 10				
Implant: IV: 10^6 Lewis Lung Carcinoma Cells					
Control (untreated)		13(1),18(3),19(2),20(1),21(1),22(1),25(1)	0/10	19.0	
B-900540	500	3(2),5(1),15(2),18(2),19(1),21(1),22(1)	0/10	16.5	-14
	335	16(1),18(3),20(2),21(1),23(3)	0/10	20.0	+5
	220	4(1),13(2),16(1),18(3),20(2),25(1)	0/10	18.0	-6
1)B-900540	1)120	2(1),3(1),4(1),6(2),7(2),8(1),10(2)	0/10	6.5	-66
2)128056	2) 10				
	1) 80	2(1),6(1),8(2),12(1),18(1),21(2),22(1),23(1)	0/10	15.0	-22
	2) 10				
	1) 53	16(1),17(1),18(3),19(2),23(1),25(2)	0/10	18.5	-3(*)
	2) 10				
	1) 35	10(1),17(1),19(1),20(1),21(2),22(1),23(2),25(1)	0/10	21.0	+10
	2) 10				
Drug Toxicity Control					
				Maximum Wt. Loss (gms)	Day Occurred
B-900540	2000	5(5),8(1),10(1)	3/10	1	5
	1000	3(2),4(1),5(1),6(2)	4/10	0	-
	500	3(2),6(1)	7/10	0	-
	335	6(3)	7/10	0	-
	220	5(1),7(1)	8/10	0	-
1)B-900540	1)120	2(3),3(1),5(1),6(2),7(2),8(1)	0/10	1	5
2)128056	2) 10				
	1) 80	8(3)	7/10	0	-
	2) 10				
	1) 53		10/10	0	-
	2) 10				
	1) 35		10/10	0	-
	2) 10				

*Highest nontoxic dose.

Experiment No. Special LL No. 290.

TABLE 3.—Evaluation of amygdalin MF and amygdalin MF plus β-glucosidase against P388 leukemia in BDF_1 mice

Implant Cells	Route	Treatment: IP; qd 1-9 days Name & NSC No.	(mg/kg/dose)	Day of Death (Number of Deaths)	41st-Day Surv./Total	Mean Life Span (day)	Mean % ILS	Median Day of Death (dying)	Median % ILS	*Approx. % Cell Kill/Dose (Logs)	*Approx. No. of Cells Alive at End of Rx
10^6	IP	Titration		10(1),11(3),12(2),13(4),14(6),15(1),16(2),17(1)	0/20	13.4		13.5			
10^5		(Doubling time		14(2),15(3),16(1),17(2),18(1),19(1)	0/10	16.0		15.5			
10^4		≈ 0.51 day)		14(1),15(3),16(2),17(4),18(5),19(1),20(1),22(1),24(1),25(1)	0/20	18.0		17.5			
10^3				16(1),17(2),18(3),19(1),20(1),21(1),22(1)	0/10	18.6		18.0			
10^2				18(1),19(1),20(2),21(1),22(2),24(2)	1/10	21.1		21.0			
10^1				18(1),22(1),38(1)	7/10	26.0		22.0			
1 Cell				20(1),21(1),23(1)	7/10	21.3		21.0			
10^6	IP	B-900540	500	12(3),13(1),14(1),15(2),16(1),17(1),18(1)		14.4	+7	14.5	+7		
		(Amygdalin	335	5(2),14(4),15(2),16(1),19(1)		15.1	+12	14.0	+3		
		MF)	220	3(1),5(1),12(1),13(1),15(2),16(2),17(1),18(1)		15.3	+14	15.0	+11(>**LD_{20})	<0.1	7.0×10^8
		1)B-900540	1)120	5(1),6(3),7(1),8(1),9(1),13(1),15(1),16(1)		9.6	-29	7.5	-45		
		2)128056	2) 10								
		(β-Gluco-	1) 80	15(4),16(2),17(2),18(2)		16.2	+20	16.0	+18		
		sidase)	2) 10								
			1) 53	13(2),14(2),15(2),16(2),18(2)		15.2	+13	15.0	+11(**)	<0.1	7.0×10^8
			2) 10								
			1) 35	13(1),14(3),15(2),16(2),17(1),18(1)		15.2	+13	15.0	+11	<0.1	7.0×10^8
			2) 10								
10^4	IP	B-900540	500	5(1),17(1),18(2),19(1),20(1),21(3),24(1)		19.9	+10	19.5	+11		
			335	3(1),6(1),18(2),19(1),20(2),21(2),22(1)		18.3	+1	19.5	+11		
			200	18(2),19(1),20(1),21(3),22(2),23(1)		20.5	+13	21.0	+20(>**LD_{20})	0.2	1.7×10^7
		1)B-900540	1)120	2(2),5(5),6(2),18(1)		10.0	-45	5.0	-72		
		2)128056	2) 10								
			1) 80	5(1),18(2),19(1),20(2),21(2),24(2)		20.6	+14	20.0	+14		
			2) 10								
			1) 53	6(2),17(1),18(1),20(3),21(1),22(2)		17.2	-5	20.0	+14(**)	0.2	6.7×10^7
			2) 10								
			1) 35	5(1),15(1),19(2),20(1),21(2),22(3)		20.1	+11	20.5	+17	0.2	3.3×10^7
			2) 10								

*Based on percent survivors (if 20% or greater); otherwise, based on median day of death (dying).
**Highest nontoxic dose (toxicity data taken from Lewis Lung No. 290).
41st-Day survivors in the titration not used in calculations. Mean life span calculated by using survivors on day 5. Median day of death calculated by using all deaths.

Experiment Nos. PS-CC 31 and 32.

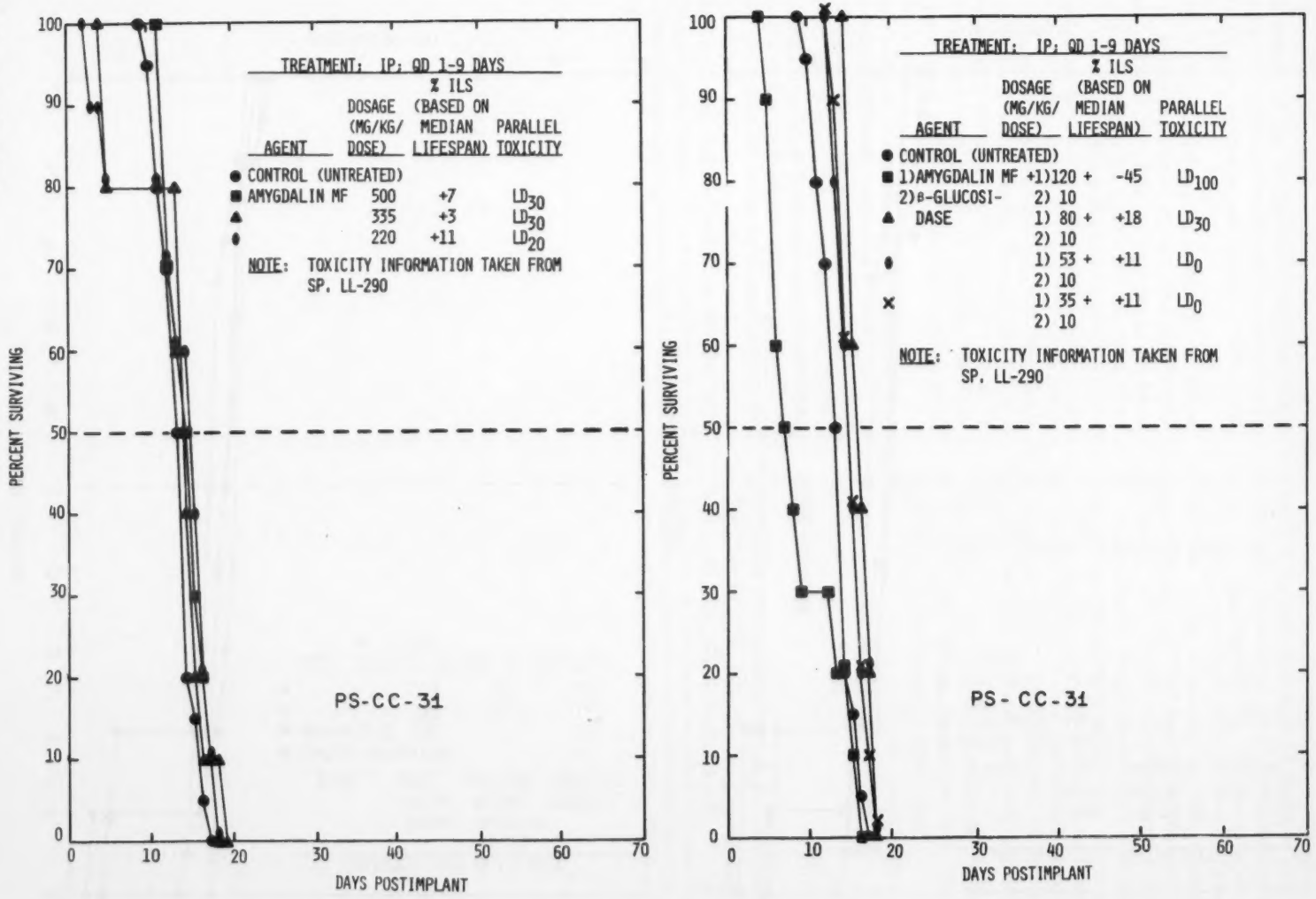


FIGURE 8.—Cumulative mortality plots of P388 leukemia (ip, 10^6 cells) treated with amygdalin MF and amygdalin MF plus β-glucosidase.

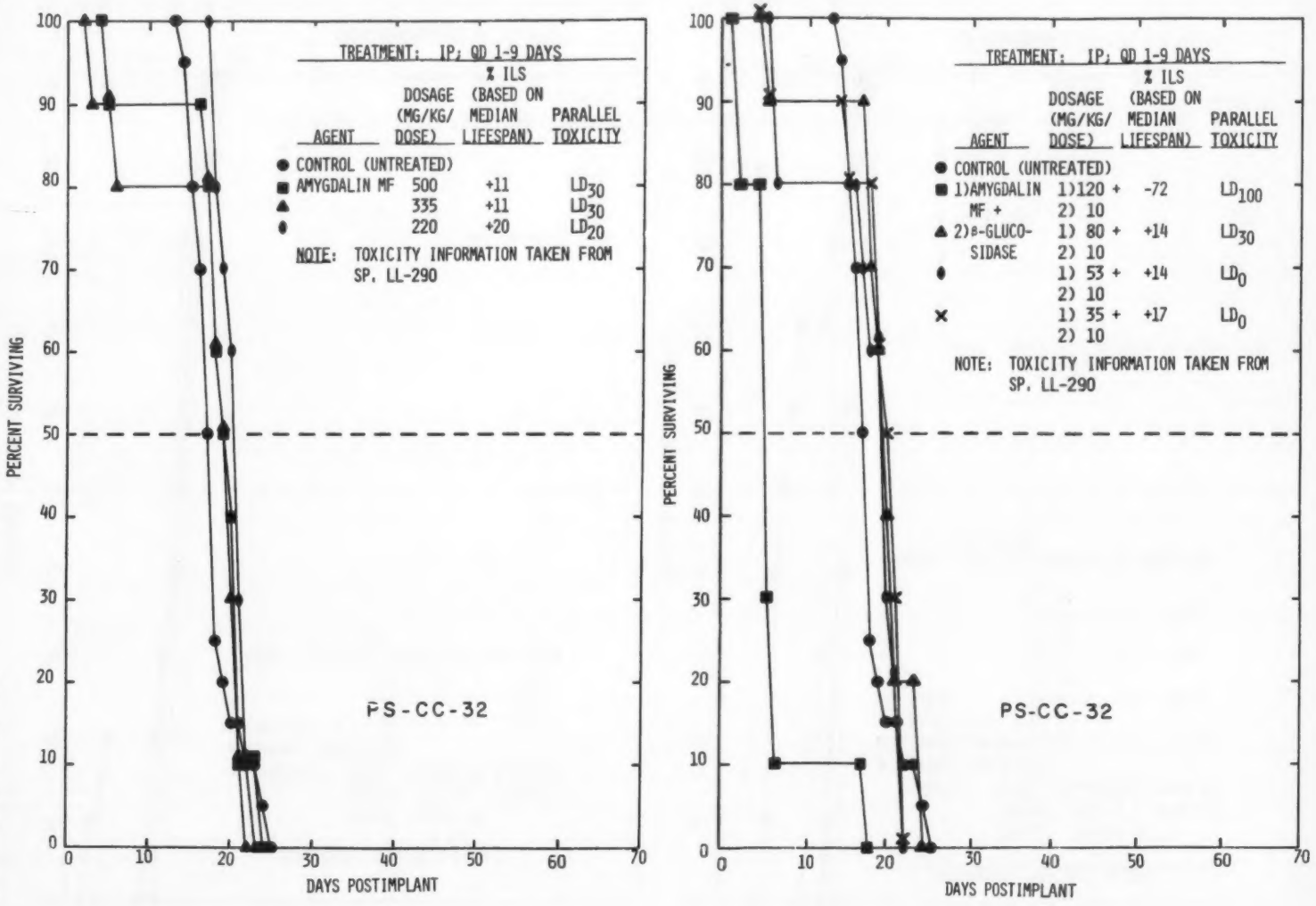


FIGURE 9.—Cumulative mortality plots of P388 leukemia (ip, 10^4 cells) treated with amygdalin MF and amygdalin MF plus β-glucosidase.

The results of the evaluation of amygdalin MF alone and in combination with β-glucosidase against the Lewis lung carcinoma are shown in table 2 and figures 4-7.

Table 2 presents the days of death for both the sc and iv implanted tumor groups and the weight change data for the drug toxicity group. At doses equal to or less than the LD20 in normal mice, there was no significant increase in median lifespan in the sc implanted group when treated with the single agent or the combination. In the iv implanted group, a 10% increase in median lifespan was observed in the group receiving 35 mg/kg of amygdalin MF plus 10 mg/kg of β-glucosidase; however, the range of deaths of the treated group (10-25 days) was essentially the same as for the untreated control group (13-25 days).

Figures 4 and 5 show the cumulative mortality of the sc and iv implanted Lewis lung groups.

Individual tumor measurements in the untreated controls and treated groups are shown in figures 6 and 7. Examination of these individual tumor measurements reveals no inhibition of tumor growth with either amygdalin MF alone or in combination with β-glucosidase.

The results of the evaluation of amygdalin MF alone and in combination with β-glucosidase against the P388 leukemia are shown in table 3 and figures 8 and 9.

Table 3 presents the days of death of mice implanted with either 10^6 or 10^4 P388 leukemia cells. In the group implanted with 10^6 cells, the greatest percent increase in median survival time (at doses ≤ LD20) was +11%, with only a 1-day increase in range of deaths over that seen in the untreated control group (an insignificant difference). In the 10^4 cell-implant group, the greatest increase in median lifespan was +17% with no increase in range of deaths over that seen in the untreated control group. Figures 8 and 9 show the cumulative mortality of the 10^6 and 10^4 P388 leukemia cell implanted mice treated with the single agent and the combination.

These data indicate that amygdalin MF alone and in combination with β-glucosidase was inactive against the P388 leukemia in BDF_1 mice.

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2. White A, Handler P, and Smith EL. Principles of Biochemistry (5th ed). New York, McGraw-Hill, 1973, p 45.
3. Schabel FM, Jr. Animal models as predictive systems. *In* Cancer Chemotherapy—Fundamental Concepts and Recent Advances. Year Book Medical Publishers, Inc., 1975, pp 323-355.

Inhibition of Antilymphoma Allograft Response in Normal and Lethally Irradiated Mice by Cyclophosphamide (NSC-26271) and Isophosphamide (NSC-109724)[1,2]

David P. Houchens,[3] Anna Iorio,[4] Anna Barzi,[4] Abraham Goldin,[3] and Enzo Bonmassar[4,5]

SUMMARY

Cyclophosphamide and isophosphamide (an analog) were tested for their effect on the antilymphoma allograft reaction in normal and lethally irradiated mice. Both drugs were effective in abrogating the classic allograft response if given 1 or 3 days before the tumor challenge. While the drugs also inhibited the hemopoietic-histocompatibility response, they were effective when administered 7 days before challenge. It is suggested that the activity of various immunosuppressive agents could be compared for their effect on the hemopoietic-histocompatibility system to select the best agent for transplantation of bone marrow or bone marrow-derived cells. Drug toxicity, as well as the effect of the drugs on humoral response and DNA synthesis, was also compared. While isophosphamide was found to be less toxic than cyclophosphamide, the effect on humoral response and DNA synthesis was comparable.

[Cancer Chemother Rep 59:967-973, 1975]

Cyclophosphamide is a widely used antineoplastic agent and there have been many reports on its immunosuppressive properties (1-5). Isophosphamide, an analog of cyclophosphamide, has been reported to show antineoplastic activity (6)[6] and significant, but somewhat reduced, immunosuppressive activity relative to cyclophosphamide (7). We investigated both of their effects against an antilymphoma allograft reaction. There are two important aspects related to the mechanism of rejection of hemopoietic-derived cells. Normal bone marrow or leukemic cells are subject to the classic allograft reaction elicited by their histocompatibility antigens. In addition, these cells express tissue-specific allo-antigens (hemopoietic-histocompatibility [Hh]) (8).[7] While the classic allograft reaction is abrogated by lethal radiation, the anti-Hh response is radioresistant (8,9). In view of the possible role played by the Hh system in the immune surveillance of virus-induced lymphomas (10), we studied the influence exerted by these immunosuppressive drugs on the Hh response. At the same time, we compared the effects of these drugs on DNA synthesis and humoral response and also compared drug toxicity.

MATERIALS AND METHODS

Animals

Mice of both sexes of the congenic-resistant strains C57Bl/10 Sc Sn Cr (abbreviated as B10, *H-2^b*, *H-1^c*), B10.129(5M) Cr (*H-2^b*, *H-1^b*), B10.A(5R) Cr (*H-2^i*), and B10.A Cr (*H-2^a*), 4-6 months old, and male (BALB/c Cr × DBA/2 Cr)F_1 (abbreviated as CDF_1, *H-2^d/H-2^d*), 2-4 months old, were used. B10.A(5R) mice differ from B10 strain for *S-D*-regions of *H-2* complex and B10.A mice for the entire *H-2* complex

[1]Received May 14, 1975; revised July 9, 1975; accepted July 22, 1975.

[2]Cyclophosphamide: CAS reg. No. 6055-19-2; *2H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Isophosphamide: CAS reg. No. 3778-73-2; 1,3,2-oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-, 2-oxide; ifosfamide.

Both drugs were supplied by the Drug Development Branch, Drug Research and Development Program (DR&DP), National Cancer Institute (NCI). Isophosphamide was also supplied by Schering Laboratories, Milan, Italy.

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[5]*Reprint requests to:* Dr. David P. Houchens, Laboratory of Experimental Chemotherapy, DR&DP, DCT, NCI, Bldg 37, Rm 5E20, National Institutes of Health, Bethesda, Md 20014.

[6]Friedman MA, and Carter SK. Clinical brochure: *N*-(2-chloroethyl)-*N'*(-2-chloroethyl)-*N'*-*O*-propylene phosphate diamide (Ifosfamide; NSC-109724). Prepared by the NCI, Bethesda, Md,Sept 1970.

[7]Bonmassar E, and Cudkowicz G. Unpublished data.

(11). The animals were obtained from the Mammalian Genetics and Animal Production Section, DR&DP, DCT, NCI, Bethesda, Maryland.

Tumors

Two radiation-induced lymphomas, LAF-17 induced in a B10.A female mouse and L5MF-22 induced in a B10.129(5M) female mouse, were obtained in 1969 through the courtesy of Dr. G. Cudkowicz. The tumors were placed in a frozen tissue bank after ten to 15 transplant generations in B10.A and B10.129(5M) female mice respectively. The tumors grow as generalized lymphomas after intravenous (iv) or intraperitoneal (ip) injection of cells into syngeneic recipients and kill 100% of the animals. Grossly enlarged spleens were used as the tumor source and the cells were dispersed in chilled medium-199 solution by teasing with a flat-end forcep. The desired amount of lymphomatous cells was injected iv in a total volume of 0.5 ml/mouse. Tumor cells removed from the tissue bank were used for experiments after a minimum of ten passages iv in syngeneic female mice. Mortality was observed for 60 days after transplantation and the presence of generalized lymphoma was confirmed at gross autopsy. During the course of serial transplantation two sublines of LAF-17 lymphoma (A and B respectively) were developed and used in our experiments. Subline B is more aggressive than subline A and kills recipient syngeneic mice in a shorter period of time.

Drugs

Cyclophosphamide and isophosphamide were dissolved in chilled saline and freshly prepared before being used. The drugs were administered ip at 0.01 ml/g of body weight.

Evaluation of ^{125}I-Labeled IUdR[8] Uptake by Spleen, Liver, and Lung In Vivo

The method of ^{125}IUdR uptake was used in these experiments to assess DNA synthesis by normal cells in the spleen, to measure cell proliferation (12), and to measure tumor cell proliferation in the spleen, liver, and lung of irradiated mice (13). Control or experimental mice were injected ip with a single dose of 5-FUdR[9] (25 μg/mouse) to decrease endogenous thymidine availability and this was followed by an ip injection of 0.25-1.0 microcurie ^{125}IUdR/mouse 1 hour later. Twenty-four hours after ^{125}IUdR administration, the animals were killed, the organs were removed, and radioactivity was determined in a well-type crystal-scintillation counter (Packard model 3002).

Measure of Mouse Antibody Response to Sheep Red Blood Cells (SRBC)

The 19S humoral antibody response of control or drug-treated mice sensitized ip with 4×10^8 SRBC was evaluated using the direct plaque-forming cells (PFC) test developed by Jerne and Nordin (14) and modified in our laboratory (15).

Total-Body Irradiation of Mice

Animals were exposed to whole body irradiation using a Westinghouse X-ray machine (Quadrocondex, 200 KV, 15 mA, 0.25-mm Cu filter and 0.55-mm Al filter, 54-cm distance, irradiation rate 137 R/minute). Lymphoma cells were injected iv 5 hours after irradiation.

RESULTS

Acute Toxicity of Cyclophosphamide and Isophosphamide

A total of 136 B10.A(5R), 112 B10, and 80 CDF_1 male mice were divided in groups of eight to 12 mice each, and injected ip with a single dose of cyclophosphamide or isophosphamide. Graded doses, from 131 to 600 mg/kg for cyclophosphamide and from 197 to 938 mg/kg for isophosphamide were used. Mortality was followed for 60 days after drug administration. The data, reported in table 1, indicate that the single dose LD50 (16) of isophosphamide is approximately half that of cyclophosphamide in B10, B10.A(5R), and CDF_1 animals.

Effect of Cyclophosphamide and Isophosphamide on Splenic DNA Synthesis "In Vivo"

The effect of single injections of cyclophosphamide or isophosphamide on DNA synthesis in a normal spleen cell population was tested in B10.A(5R) female mice; 120 mice (26-30 g) were divided into 12 groups of ten animals each and treated ip with graded doses of cyclophosphamide or isophosphamide. One day later 5-FUdR and ^{125}IUdR (0.25 microcurie/mouse) were administered ip and splenic uptake of the labeled precursor was measured 24 hours after ^{125}IUdR injection. The data, reported in figure 1, indicate that both cyclophosphamide and isophosphamide strongly inhibited the proliferative activity of the splenic cell population. The inhibition was

[8]IUdR: NSC-39661;CAS reg. No. 54-42-2; uridine, 2'-deoxy-5-iodo-.
[9]5-FUdR: NSC-27640; uridine, 5-fluoro-2'-deoxy-.

TABLE 1.—Acute toxicity of single ip injections of cyclophosphamide or isophosphamide in B10, B10.A(5R), and CDF_1 male mice

Mouse strain	Body weight range (g)	Drug	LD50* (mg/kg)	Confidence limits (mg/kg)	Slope
B10	24-28	Cyclophosphamide	225	179-283	1.5
		Isophosphamide	505	412-619	1.43
B10.A(5R)	26-30	Cyclophosphamide	256	240-274	1.19
		Isophosphamide	465	404-535	1.33
CDF_1	22-24	Cyclophosphamide	295	234-378	1.67
		Isophosphamide	555	514-599	1.08

*Calculated according to Litchfield and Wilcoxon (*from* ref 16).

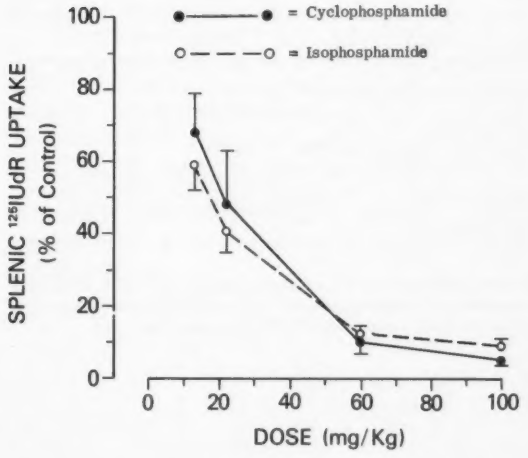

FIGURE 1.—Effect of cyclophosphamide and isophosphamide on splenic DNA synthesis in B10.A(5R) mice. Drugs were administered ip; ^{125}IUdR uptake in spleen was measured 48 hrs later.

related to the dose injected and little or no difference was found between cyclophosphamide and isophosphamide when given at the same dose levels.

Effect of Pretreatment With Single Doses of Cyclophosphamide or Isophosphamide on Humoral Response (19S) of Mice Against SRBC

B10.A(5R) male mice were treated ip with graded doses of cyclophosphamide or isophosphamide and 1 day later were injected with SRBC. Four days after SRBC administration, PFCs were assayed and the data, reported in figure 2, indicate a direct relation between the dose of drug used for pretreatment and the depression of 19S antibody response. No statistically significant differences were found between the immunodepressive effects of cyclophosphamide and isophosphamide. The kinetics of PFC response to SRBC were then studied in B10.A(5R) male mice, untreated or treated 1 day before SRBC with cyclophosphamide or isophosphamide (50 mg/kg ip). The data, reported in figure 3, indicate the following: the peak of PFC response was on Day 4 in untreated control mice, whereas a shifting of peak was observed in cyclophosphamide-treated animals, as already reported in mice treated with 5-azacytidine[10] (17); no significant differences of PFC response were found in mice pretreated with cyclophosphamide or isophosphamide on Days 2 and 4 after SRBC administration. On Day 6 after SRBC, the PFC response was higher in cyclophosphamide-pretreated mice whereas on Day 8 the response was higher in isophosphamide-treated animals. In any case, the differences were minimal although statistically significant (fig 3).

Effect of Pretreatment With Single Doses of Cyclophosphamide or Isophosphamide on Antilymphoma Allograft Response of Nonirradiated Recipient Mice

Syngeneic B10.A or congenic-resistant B10 male mice, differing for the entire *H-2* complex, were injected with two sublines (A and B) of B10.A lymphoma LAF-17. The LAF-17(A) subline was less aggressive than the LAF-17(B) subline, as indicated by the longer survival time of syngeneic B10.A recipient mice upon iv challenge despite the use of a higher inoculum (table 2). The sublines with two different growth rates were used in order to test the drug-induced immunodepression of the allograft response at two different sensitivity levels. As reported in table 2, allogeneic B10 mice challenged with 10^7 cells of subline A died with generalized lymphoma if they were pretreated with 200 mg/kg of

[10]5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-.

either drug. All B10 mice, either non-pretreated or depressed by cyclophosphamide and isophosphamide at 72 or 120 mg/kg, rejected the tumor. On the other hand, when the more aggressive LAF-17(B) subline was used, a large proportion of mice pretreated with

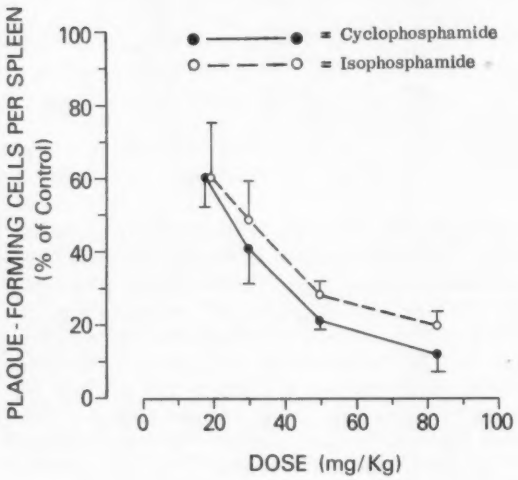

FIGURE 2.—Effect of cyclophosphamide and isophosphamide on humoral response of B10.A(5R) to SRBCs. Drugs were administered ip as indicated; SRBCs were given 1 day later and the plaque assay was performed 4 days after the injection of SRBCs.

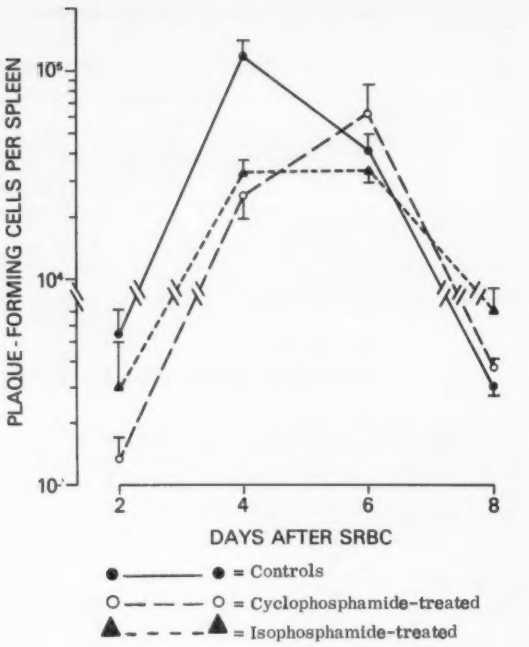

FIGURE 3.—Kinetics of 19S antibody production in B10.A(5R) mice treated with cyclophosphamide and isophosphamide. Drugs were injected ip (50 mg/kg) and SRBCs were given 1 day later.

TABLE 2.—Inhibitory effects of single treatment with cyclophosphamide or isophosphamide on the antilymphoma allograft reaction of B10 male mice challenged with 10^7 or 10^6 cells of two sublines of B10.A lymphoma LAF-17 1 day after drug treatment

Recipient pretreatment (Day -1)			Challenge (Day 0)			
			Iv LAF-17 (subline A) (10^7 cells)		Iv LAF-17 (subline B) (10^6 cells)	
Strain (male mice)	Drug	Ip dose (mg/kg)	MST*	D/T†	MST	D/T
B10.A	None	—	9	6/6	8	6/6
	Cyclophosphamide	200	9	6/6	NT‡	NT
	Isophosphamide	200	9	6/6	NT	NT
B10	None	—	—	0/6	—	1/8
	Cyclophosphamide	72	—	0/6	15	4/7
		120	—	0/6	9	7/7
		200	12	6/6	8	8/8
	Isophosphamide	72	—	0/6	12	4/7
		120	—	0/6	9	6/8
		200	12	5/7	9	8/8

*MST = median survival time in days.
†D/T = No. of animals dead/total animals tested.
‡NT = not tested.

cyclophosphamide and isophosphamide at any dose level died with generalized lymphoma. No difference was found in the inhibition of antilymphoma allograft response by the same doses of cyclophosphamide and isophosphamide. The study was extended to the L5MF-22 tumor system and the duration of drug-induced depression of the allograft response was determined. CDF_1 hybrid mice *(H-2^d/H-2^d)* were treated with cyclophosphamide or isophosphamide 1, 3, or 7 days before the iv injection of 10^7 cells of allogeneic L5MF-22 lymphoma (of *H-2^b* origin). Host-tumor incompatibility was multiple, including the entire *H-2* complex and some minor histocompatibility loci. The survival data (table 3) indicate that an effective allograft response was present in mice 7 days after treatment with both drugs at a dose of 200 mg/kg. When graded doses of the immunodepressive agents were given 3 days before challenge, no depression was found after 72 or 120 mg/kg. At the dose of 200 mg/kg, isophosphamide and cyclophosphamide clearly inhibited the allograft response. When the agents were given 1 day before challenge the allograft response was depressed somewhat more effectively by cyclophosphamide than isophosphamide at equal doses. (Roughly 200 mg/kg of isophosphamide was equivalent to 120 mg/kg of cyclophosphamide.)

Effect of Pretreatment With Single Doses of Cyclophosphamide or Isophosphamide on Antilymphoma Allograft Response of Lethally Irradiated Recipient Mice

CDF_1 male mice were treated with 100 or 200 mg/kg of the two drugs. Seven days later untreated syngeneic B10.129(5M) controls and untreated or drug-pretreated CDF_1 mice were lethally irradiated with 700 R and 5 hours later challenged iv with 10^5 cells of L5MF-22 lymphoma *(H-2^b)*. Five days later all mice were treated ip with 5-FUdR and ^{125}IUdR (1 microcurie/mouse) and 1 day later the spleen, liver, and lung were collected from each mouse and the drug uptake was counted. The mean uptake of ^{125}IUdR per organ, expressed in percent of the amount of injected isotope is reported in figure 4. As indicated by the extent of decreased uptake of the ^{125}IUdR, proliferation of L5MF-22 was markedly inhibited in the organs of nondrug-treated allogeneic CDF_1 mice. Pretreatment with both drugs at 100 mg/kg partially diminished the percent of uptake of ^{125}IUdR in the liver and lung, but not in the spleen. In mice pretreated with 200 mg/kg of the two drugs, there was uptake of ^{125}IUdR in spleen, liver, and lungs, apparently reflecting tumor growth in these organs (fig 4).

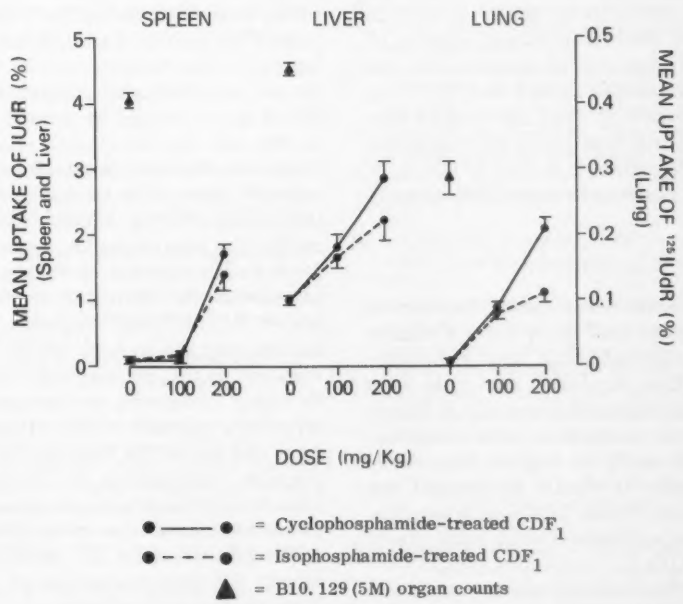

FIGURE 4.—Effect of cyclophosphamide and isophosphamide on DNA synthesis as a measure of L5MF-22 tumor growth in various organs of mice. 10^5 Tumor cells were administered iv 5 hours after lethal irradiation and 7 days after drug administration; 5 days later 1 microcurie/mouse of ^{125}IUdR was injected and the organs were harvested and counts were performed. Counts are in % of isotope injected.

TABLE 3.—Inhibitory effects of single treatment with cyclophosphamide or isophosphamide on the antilymphoma allograft reaction of CDF_1 male mice challenged (Day 0) with 10^7 cells of L5MF-22 lymphoma (of B10.129[5M] origin) at various intervals after drug treatment*

Day of drug treatment	Ip dose (mg/kg)	Cyclophosphamide		Isophosphamide	
		MST†	D/T†	MST	D/T
-7	200	—	2/8	—	1/7
-3	72	—	0/8	—	0/8
	120	—	0/8	—	0/8
	200	—	4/8	12.5	5/8
-1	72	—	0/8	—	0/8
	120	10	7/8	—	3/8
	200	6.5	8/8	10.5	8/8

*D/T = 1/8 in CDF_1 mice challenged with L5MF-22 and not pretreated with drugs. All B10.129(5M) syngeneic mice challenged with the same lymphoma died with MST = 7 days.
†See table 2 for explanation of abbreviations.

A bioassay of spleens from these animals was performed to confirm the growth of tumor in the suppressed animals. The results are seen in table 4. The median survival times in recipients of spleens from nondrug-pretreated CDF_1 mice were considerably longer than those of recipients of spleens from syngeneic control mice, indicating the transfer of fewer viable tumor cells than from the former. When spleens were bioassayed from CDF_1 mice that received either drug at the 200-mg/kg level, the median survival times were reduced, indicative of more extensive growth of the tumor in the donor animals. It should be pointed out that recipients of spleens of nondrug-treated CDF_1 donors died considerably later than recipients of spleens from B10.129(5M) or drug-treated CDF_1 donors. In fact, the median survival time would indicate that there were approximately only 10^2 cells present in the fraction of spleen given from the nondrug-treated CDF_1 donors.

DISCUSSION

The comparison of toxicities of cyclophosphamide and isophosphamide shows that the ip LD50 is considerably higher for isophosphamide than for cyclophosphamide in all three strains of mice that were tested. This is in agreement with the study of Harrison and Fuquay (7) who found that in mice isophosphamide is tolerated orally at higher dose levels than cyclophosphamide. It should be pointed out that a recent report of clinical trials with isophosphamide has presented evidence of some toxic effects observed in patients (18).

The results of the effects of cyclophosphamide and isophosphamide on DNA synthesis indicate that normal cell proliferation in the spleen is grossly affected to the same extent by both agents in a dose-response study. Since isophosphamide is less toxic than cyclophosphamide, the lethal effect is apparently not strictly related to the inhibition of DNA synthesis since the DNA synthesis was equally affected. Also, the effect of the agents on 19S antibody production against SRBC was comparable. Pretreatment of mice with cyclophosphamide and isophosphamide to monitor the effect on the classic allograft response showed that the drugs had approximately the same activity at equal doses. While both drugs were effective in abrogating the classic allograft response if given 1 or 3 days before the tumor challenge, the animals that received the drug 7 days before the challenge had recovered immunologically. The measurement of the effect on the Hh system also indicated comparable inhibition by the two drugs. It should be pointed out that in contrast to the test on the classic allograft response, the drugs are effective on the Hh response when administered 7 days prior to challenge. This difference in the timing of drug administration could provide a method for separating the classic allograft response from the Hh response. Several studies have presented evidence for differential effects of cyclophosphamide on B- and T-cell responses (1,4,19-21). Further studies may aid in determining the actual cell type involved in the Hh response. Our data confirm the finding of Cudkowicz and Bennett (8) that cyclophosphamide is capable of inhibiting the Hh response in mice, and extend the findings for the analog isophosphamide. Comparison of the activity of various immunosuppressive drugs on the Hh system will lead to further elucidation of aspects of the Hh system and therefore could be useful in the selection of agents for transplantation of bone marrow or of bone marrow-derived cells.

In conclusion, cyclophosphamide and isophosphamide showed approximately the same activity in inhibiting humoral responses and antilymphoma

TABLE 4.—Bioassay of spleens from lethally irradiated syngeneic (B10.129[5M]) or allogeneic (CDF_1) male mice transplanted with L5MF-22 lymphoma

Donor		Recipient†					
		Fraction of donor spleen transferred iv‡ on Day 5 after challenge					
		1/50			1/5		
Mouse strain	Treatment before challenge*	MST§	D/T§	Range	MST	D/T	Range
B10.129(5M)	—	7.5	4/4	7-8	7	5/5	7
CDF_1	—	14.5	4/4	12-16	12	4/4	11-12
CDF_1	Cyclophosphamide (200 mg/kg ip)	10.5	4/4	10-11	10	4/4	9-11
CDF_1	Isophosphamide (200 mg/kg ip)	10	4/4	10	9	4/4	9-10

*Drugs were administered 7 days before total-body irradiation (700 R) and L5MF-22 challenge (10^5 cells iv).

†C57Bl/6 male mice treated with 150 mg/kg of cyclophosphamide ip 5 hrs before cell transfer in order to inhibit the presumed weak allograft response; a tumor titration with L5MF-22 tumor (iv) gave the following survival data: 10^6, MST = 8, D/T = 7/7; 10^4, MST = 11, D/T = 8/8; 10^2, MST = 13, D/T = 8/8. No difference was found in survival times of nonimmunodepressed C57Bl/6 or syngeneic B10.129(5M) mice transplanted with the same number of lymphoma cells.

‡Spleen cell suspension in medium-199, injected in 0.5 ml/mouse.

§*See* table 2 for explanation of abbreviations.

allograft reactions, either classic or Hh. These observations are consistent with the finding that equal dosage of the two drugs resulted in equal inhibition of DNA synthesis in the spleen. Since inhibition of DNA synthesis was comparable for both drugs, other pharmacologic factors would appear to be responsible for the diminished host toxicity of isophosphamide.

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Murine Neuroblastoma: Further Evaluation of the C1300 Model With Single Antitumor Agents[1,2,3]

J. Z. Finklestein, K. Tittle, R. Meshnik, and J. Weiner[4,5]

SUMMARY

The murine C1300 neuroblastoma model has been evaluated as a possible model for children with widespread metastatic disease. Drug toxicity studies were conducted in adult A/J mice with various doses of antitumor agents. Adriamycin, BCNU, bleomycin, guanazole, acronycine, isophosphamide, DTIC, ICRF-159, cyclophosphamide, vincristine, and vinblastine were administered intraperitoneally to random groups of normal mice. After identification of appropriate doses, chemotherapy studies were conducted with various regimens of drugs. Chemotherapy was administered to adult A/J mice when their subcutaneously implanted tumors measured 1.0-1.7 cm in diameter. Antitumor drugs can be classified into three groups according to drug efficacy. BCNU, cyclophosphamide, and isophosphamide were extremely active. Cytosine arabinoside was reported to be active against this murine tumor in a previous publication. Drugs with minimal activity which deserve further evaluation included adriamycin, guanazole, ICRF-159, DTIC, and vinblastine. Inactive drugs were acronycine, bleomycin, 5-fluorouracil, and vincristine. These experiments suggest that children with metastatic neuroblastoma may respond to cyclophosphamide, isophosphamide, and BCNU, while DTIC, adriamycin, ICRF-159, guanazole, and the vinca alkaloids may also be effective. The results suggest that agents selected by the C1300 model should be given adequate clinical trials.

[Cancer Chemother Rep 59:975-983, 1975]

A review of the impact of chemotherapy on the treatment of metastatic neuroblastoma has revealed a lack of significant improvement in the survival of children with stage IV disease (1). The tumor does not respond in the same manner as acute lymphocytic leukemia, Wilms' tumor, and rhabdomyosarcoma to the newer forms of drug therapy for children with these neoplastic diseases (2-4). The classic animal tumor systems used for the determination of the efficacy of antitumor agents include one or more of the following: murine L1210 leukemia, murine P388 leukemia, murine B16 melanoma, murine Lewis lung carcinoma, rat Walker 256 carcinosarcoma, and murine Carcinoma 755 (5). These primary screening tumors often select drugs which are found to be active in other tumors in children, yet they do not specifically test for neuroblastoma. Many agents active in these model systems are inactive in children with stage IV disease (6-9).

The relevance of the C1300 murine neuroblastoma system as a model for the study of the disease in children has been under investigation in our laboratory (10). We have shown that cyclophosphamide, BCNU, and cytosine arabinoside prolong the lifespan of adult A/J mice with palpable disease. Actinomycin D, methotrexate, vincristine, 6-hydroxydopamine, papaverine, bretylium tosylate, butyric acid, and cyanocobalamin were inactive at the doses used (10,11). Cyclophosphamide is believed to be the only agent to which a significant number of children with metastatic disease will respond (12), and the sensitivity of the murine neoplasm to this agent appears to correlate with observations in children with active disease. There is a need for additional active agents for children with widespread disease, and further investigations with the C1300 model have been performed

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[3]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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in the hope that the results will be helpful to clinicians in the design of clinical regimens for the human tumor.

MATERIALS AND METHODS

The murine model has been described previously, and thus will be briefly summarized. Adult A/J mice (more than 8 weeks of age) were obtained from Jackson Laboratories and, after a 7-10-day period of quarantine, were randomized and admitted into the study. Murine neuroblastoma cells supplied by Drs. Harvey Herschman and John Byfield (UCLA School of Medicine) were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum. Tumor induction was performed by inoculation of 10^6 viable tumor cells subcutaneously (sc) in the interscapular area.

Quality control procedures included monitoring of environmental factors by leukocyte counts and observation of random groups of mice (average weight, 20 g). Reproducibility of results was assessed by injecting five random mice on alternate experiments with murine neuroblastoma cells and monitoring the effect of cyclophosphamide chemotherapy using 2.5 mg/10 g of body weight as a positive control.

Drug toxicity studies were conducted with adult A/J mice using various doses of the antitumor agents. Adriamycin, BCNU, bleomycin, guanazole, isophosphamide, and DTIC were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment (DCT), NCI; ICRF-159 was supplied by Dr. Harry Wood, Drug Research and Development, DCT, NCI; and acronycine was supplied by Dr. Michael Sweeney of the Eli Lilly Company. Other drugs used in the study included cyclophosphamide, vincristine, and vinblastine. All drugs were administered in a 0.2-1.0 ml aliquot intraperitoneally (ip) to random groups of normal adult mice. Initial experiments utilized three doses chosen to represent the lower, middle, and higher values of the dose range with three animals assigned to each dose. After identification of a lethal dose, intermittent doses were administered to between six and 12 animals per dose to discern smaller differences in toxicity. Chemotherapy studies were conducted with various doses of drug, which were administered when the sc implanted tumor measured 1.0-1.7 cm in diameter. Animals in treatment and control groups were matched for tumor size prior to the administration of antitumor agents. Drug regimens always included the approximate LD10 dose, the highest nontoxic dose, and a lower dose, allowing for the possibility that antitumor therapy is more toxic in animals with growing neoplasms than in their nontumor-bearing controls.

Statistical analysis of toxicity was done comparing sequentially the proportions of the groups dying at adjacent levels. The Fisher exact test of contingency tables was used. In the analysis of chemotherapeutic effects, the median for the control animals was compared with the median for each non-zero dose. Significant change from control was identified using the Fisher exact test of contingency tables (13).

RESULTS

Drug toxicity results are outlined in table 1. Dose levels associated with significant toxicity are identified. The Fisher exact test was used to compare toxicity experience at all lower doses (pooled) with that observed at the designated dose. Results are reported at the 5% level of significance because of the multiple testing performed. The criterion of significance used in this multitest situation was $P<0.001$.

It was not possible to formulate an injectable solution with acronycine, although various solubilizing techniques—emulfluor (GAF), tween 80 (Sigma), and ultrasonification—were tried. Therefore, a single combined toxicity and chemotherapy experiment was performed to ensure usage of a toxic dose. The effects of chemotherapy on palpable, sc implanted neuroblastoma in adult A/J mice are tabulated in table 2. Significant differences in lifespan between control and treatment groups are identified.

The following criteria were utilized to group antitumor agents according to drug efficacy. One group of drugs consisted of those agents which caused a greater than 50% reduction in tumor volume and significantly increased animal lifespan by 50% or more. BCNU, cyclophosphamide, and isophosphamide met these criteria. A second group of drugs failed to prolong animal lifespan 50% or more. These latter agents were acronycine, bleomycin, 5-fluorouracil (5-FU), and vincristine. The third group of agents fell between the above two categories in that they did not cause tumor regression but did prolong animal lifespan 50% or more. These latter drugs were adriamycin, guanazole, ICRF-159, DTIC, and vinblastine (table 3).

DISCUSSION

The chemotherapy studies with cyclophosphamide, DTIC, BCNU, and vincristine were, in part, continuations of pilot experiments previously reported with this murine system (10). The evaluations of acronycine, adriamycin, bleomycin, 5-FU, guanazole, isophosphamide, ICRF-159, and vinblastine

TABLE 1.—Drug toxicity of antitumor agents administered to adult A/J mice

Drug	Administered on—	Dose ip (mg/10 g of body wt/injection)	No. of deaths/ No. of animals treated	Mean time to death (days)
Acronycine	Day 1	1.0	0/17	—
		2.0	0/6	—
		5.0	1/5	14
		10.0	1/8	7
Adriamycin	Day 1	0.001-0.05	0/9	—
		0.1	1/9	14
		0.13	10/12	13.2*
		0.16-0.5	30/30	7.6
Adriamycin	q3h × 3 × 1 day	0.0015-0.025	0/36	—
		0.03	1/9	21
		0.15	3/3	8*
Adriamycin	Days 1-5	0.002-0.01	0/9	—
		0.013	1/12	15
		0.016	1/12	12
		0.02	6/9	16.3*
		0.10-0.20	6/6	5.5
Adriamycin	Days 1, 3, 5	0.02	0/3	—
		0.03	1/9	15
		0.04	3/9	10.3
		0.05	3/3	8.3*
BCNU	Day 1	0.18	2/9	23
		0.2	0/6	—
		0.24	0/10	—
		0.3	4/6	15
		0.32	4/10	14
		0.4	11/16	13*
BCNU	Days 1-3	0.05-0.10	0/12	—
		0.2	2/6	11
Bleomycin	Day 1	0.02-0.2	0/12	—
		0.5	2/12	12
		0.8	0/12	—
		1.0	3/9	11.8*
		1.5	3/3	10.7
Bleomycin	Days 1-5	0.01	0/3	—
		0.02	1/3	12
		0.1	0/9	—
		0.13	2/12	14.5
		0.16	2/12	12.5
		0.2	4/6	13.5*
		1.0	3/3	7
Bleomycin	Days 1,5,9	0.01-0.1	0/6	—
		0.2	1/9	14
		0.5	11/12	12*
		0.8	10/12	12.4
		1.0	9/9	12.7
Cyclophosphamide	Day 1	2.3	1/6	18.0
		2.5	0/6	—
		2.8	1/6	11.0
Cyclophosphamide	Days 1-5	0.1-0.3	0/9	—
		0.4	1/9	16
		0.43	3/6	14*
		0.46	2/6	17.5
		0.5	2/9	15
		1.0-2.0	9/9	9.4
Cyclophosphamide	Days 1, 5, 9	0.2	0/6	—
		0.4	5/12	13.6
		0.5	2/6	17.5

TABLE 1.—Drug toxicity of antitumor agents administered to adult A/J mice—Continued

Drug	Administered on—	Dose ip (mg/10 g of body wt/injection)	No. of deaths/ No. of animals treated	Mean time to death (days)
		0.6	1/12	18.0
		0.8	3/12	13.0
		1.0	5/12	11.8
		1.25	0/6	—
		2.0	3/6	12
		3.0	6/6	11.2
5-FU	Day 1	0.1-0.3	0/21	—
		0.6	2/12	9
		1.0	4/9	10.8*
		5.0-10.0	7/7	9
5-FU	Days 1-5	0.05	0/9	—
		0.15	3/18	12.7
		0.3-5.0	27/27	8.4*
5-FU	Days 1, 5, 9	0.08-0.25	0/21	—
		0.4	1/6	12
		0.5	6/12	10.2*
		0.8-8.0	15/15	9
Guanazole	Day 1	10-30	0/9	—
		40.0	1/9	2
		45.0	3/12	2
		50.0	2/3	4
		55-80	27/27	2.8*
Guanazole	Days 1-5	5-10	0/12	—
		12.0	1/11	5
		17.0	6/12	9*
		20-30	12/12	8.5
Guanazole	Days 1, 5, 9	30.0	0/9	—
		35.0	1/12	2
		40.0	4/15	9
		45.0	5/6	6.4*
		60-80	6/6	3.3
Guanazole	Days 1, 3, 5	20-35	0/15	—
		40.0	1/6	5
		45.0	5/7	5.8*
		50.0	6/6	5.7
ICRF-159	Day 1	0.5-3.0	0/18	—
		3.5	4/6	10*
		4.0	5/9	8.6
		8.0	2/3	10
		10.0	2/3	7
		12.0	3/3	7
ICRF-159	Days 1-5	0.01-0.25	0/17	—
		0.50	1/9	12
		0.75	5/11	9.6*
		1.0	3/3	10
ICRF-159	Days 1, 5, 9	2.0-2.5	0/21	—
		3.0	1/12	12
		4.0	3/9	10*
		8.0-10.0	6/6	6.3
Isophosphamide	Day 1	1.0-4.0	0/15	—
		5.0	3/12	6.7
		6.0	11/12	6.5*
		7.0-8.0	9/9	5.1
Isophosphamide	Days 1-5	0.05-2.5	0/15	—
		3.0	2/6	12
		3.5	2/6	16.5
		4.0-4.5	9/9	7.9*

TABLE 1.—Drug toxicity of antitumor agents administered to adult A/J mice—Continued

Drug	Administered on—	Dose ip (mg/10 g of body wt/injection)	No. of deaths/ No. of animals treated	Mean time to death (days)
Isophosphamide	Days 1, 5, 9	0.5-2.5	0/15	—
		3.0	10/18	17.9
		3.5	9/12	20.1
		4.0	10/21	10.3*
DTIC	Days 1-5	0.5-1.0	0/12	—
		1.25	3/12	13.6
		1.5	3/12	14.7
		2.0-4.0	12/12	7.8*
DTIC	Days 1, 5, 9	1.0-1.5	0/12	—
		2.0	6/6	16.5*
Vinblastine	Day 1	0.01-0.03	0/14	—
		0.04	1/6	10
		0.05	3/6	7.3*
		0.06	9/11	6.6
		0.10	3/3	9.3
Vinblastine	Days 1-5	0.0025-0.006	0/12	—
		0.007	1/6	24
		0.0075	1/3	12
		0.008-0.01	15/15	8.6*
Vinblastine	Days 1, 3, 5	0.0025-0.004	0/9	—
		0.005	2/3	4
		0.006	0/6	—
		0.0075	0/3	—
		0.008	1/6	5
		0.01	2/9	6.5
		0.015	6/6	6.2*
Vincristine	Days 1-5	0.001	0/3	—
		0.002	2/9	11
		0.003	1/9	12
		0.004	8/15	10.7*
		0.005-0.01	30/30	8.7
Vincristine	Days 1, 3, 5	0.001-0.00125	0/9	—
		0.0025	1/9	8
		0.003	0/6	—
		0.004	0/6	—
		0.005	4/15	9.8*
		0.006	4/6	9.5
		0.0075	5/6	8.4
		0.01	3/3	8

*Test of significance: dose identified vs. lower dose pooled. *See* Results section in the text.

represent new drug studies on the murine neuroblastoma model. These experiments suggest that the model is extremely sensitive to alkylating agents such as cyclophosphamide, isophosphamide, and BCNU. In addition, selected regimens of adriamycin, guanazole, ICRF-159, DTIC, and the vinca alkaloids demonstrated borderline activity. The preferred sensitivity to cell-cycle nonspecific agents is in keeping with autoradiographic studies of the murine neoplasm that have revealed a relatively short DNA synthesis "S" phase with a ratio of DNA synthesis time to the total generation time of 0.38 (14). The only known cell-cycle specific agents which demonstrate activity in the murine neoplasm are cytosine arabinoside (10) and guanazole. Their activity may be related to peculiar characteristics of the agents or the tumor.

The goal of the investigations with the murine C1300 neuroblastoma is to utilize information gained from the model to develop an organized approach to the treatment of children with metastatic disease. To be a true chemotherapeutic predicator, the ideal animal model should respond to all those agents active in its specific human counterpart. The

TABLE 2.—Effect of chemotherapeutic agents on neuroblastoma in A/J mice with sc tumors

Drug	Administered on—	Dose ip* (mg/10 g of body wt/injection)	No. of mice	Survival time in days		
				Range	Median	%ILS†
Acronycine	Day 1	Control	9	5-14	10	—
		1.0	10	5-19	9	N
		5.0	10	5-17	13.5	N
		10.0	10	3-19	8.5	N
Adriamycin	Day 1	Control	14	8-16	10	—
		0.05	9	8-16	15	50%
		0.08	8	8-15	9	N
		0.10	16	5-26	12	N
		0.12	6	7-12	9	N
Adriamycin	q3h × 3 × 1 day	Control	9	8-16	11	—
		0.02	10	10-16	15	N
		0.025	10	8-16	12.5	N
		0.03	10	8-12	10	N
Adriamycin	Days 1-5	Control	9	8-16	11	—
		0.01	10	8-12	11	N
		0.013	10	8-16	12.5	N
		0.017	10	8-12	8	N
Adriamycin	Days 1,3,5	Control	8	4-15	12	—
		0.02	10	5-12	8	N
		0.03	10	3-10	8	N
		0.04	10	5-12	9	N
BCNU	Day 1	Control	13	7-24	10	—
		0.18	13	13-84	20	100%
		0.24	13	13-29	20	100%‡
BCNU	Days 1-3	Control	10	5-22	9.5	—
		0.10	10	8-29	18	89.4%
		0.15	10	9-29	22	132%‡
		0.20	10	8-12	8.5	N
Bleomycin	Day 1	Control	19	5-22	8	—
		0.1	9	5-10	8	N
		0.15	8	5-9	8	N
—	—	0.2	10	5-24	10	N
		0.3	10	3-15	8	N
		0.5	10	3-10	8	N
		0.6	10	6-10	8	N
Bleomycin	Days 1-5	Control	25	5-22	10	—
		0.06	9	9-22	9	N
		0.08	10	9-18	9.5	N
		0.10	10	6-17	11	N
		0.13	10	6-26	10	N
		0.16	10	5-12	10	N
Bleomycin	Days 1,5,9	Control	19	5-22	8	—
		0.075	9	5-9	8	N
		0.10	10	5-17	9.5	N
		0.15	10	3-10	5	N
		0.20	10	3-11	6.5	N
Cyclophosphamide	Day 1	Control	24	1-12	8	—
		2.0	7	8-29	22	175%‡
		2.5	29	3-30	24	200%‡
Cyclophosphamide	Days 1-5	Control	19	5-22	10	—
		0.2	9	8-29	15	50%
		0.3	10	9-35	30	200%‡
		0.4	10	6-9	9	N
		0.5	10	5-16	9.5	N
Cyclophosphamide	Days 1,5,9	Control	15	3-15	8	—
		0.1	10	8-24	13.3	66.2%
		0.2	10	5-23	8	N

TABLE 2.—Effect of chemotherapeutic agents on neuroblastoma in A/J mice with sc tumors—Continued

Drug	Administered on—	Dose ip* (mg/10 g of body wt/injection)	No. of mice	Survival time in days		
				Range	Median	%ILS†
		0.4	20	3-31	13.5	66.2%
		0.6	10	8-33	30	275%‡
5-FU	Day 1	Control	20	5-14	8	—
		0.3	10	8-9	8	N
		0.4	10	8-8	8	N
		0.5	20	5-23	9	N
		0.6	10	9-13	11	N
5-FU	Days 1-5	Control	10	4-15	9	—
		0.05	10	6-10	9	N
		0.10	10	4-19	9	N
		0.15§	10	6-15	9	N
5-FU	Days 1,5,9	Control	10	8-10	8	—
		0.2	10	5-10	8.5	N
		0.3	10	5-25	8.5	N
		0.4	10	8-10	8	N
Guanazole	Day 1	Control	19	3-26	8	—
		30	9	3-12	8	N
		35	10	1-38	10.5	N
		40	10	1-31	12	50%
		45	10	1-22	3.5	N
Guanazole	Days 1-5	Control	10	6-26	8	—
		10	10	3-24	9.5	N
		12	10	6-29	13	53%
		14	10	5-23	8	N
Guanazole	Days 1,5,9	Control	10	8-12	8	—
		25	10	8-21	9.5	N
		30	9	8-36	15	87.5%‡
		35	9	8-33	12	50%‡
Guanazole	Days 1,3,5	Control	19	3-12	8	—
		20	9	3-26	10	N
		25	9	3-10	8	N
		30	9	3-22	10	N
		40	10	5-15	8	N
ICRF-159	Day 1	Control	10	5-14	11	—
		2.5	10	5-14	10	N
		3.0	10	7-12	10	N
		3.5	10	7-26	12	N
ICRF-159	Days 1-5	Control	10	5-39	9	—
		0.25	9	7-14	9	N
		0.50	9	9-30	16	77.8%
		0.75	9	9-12	11	N
ICRF-159	Days 1,5,9	Control	10	5-18	12	—
		2.5	10	6-12	8	N
		3.0	10	6-10	8.5	N
		3.5	10	8-26	9	N
Isophosphamide	Day 1	Control	9	5-12	8	—
		3.0	10	8-29	18	125%
		4.0	10	8-29	18	125%
		5.0	10	5-36	26	225%
Isophosphamide	Days 1-5	Control	18	4-15	10	—
		1.0	10	8-37	10	N
		1.5	10	3-36	19.5	95%
		2.0	10	8-41	9	N
		2.5	10	8-39	8	N
		3.0	10	5-10	8	N

TABLE 2.—Effect of chemotherapeutic agents on neuroblastoma in A/J mice with sc tumors—Continued

Drug	Administered on—	Dose ip* (mg/10 g of body wt/injection)	No. of mice	Survival time in days		
				Range	Median	%ILS†
Isophosphamide	Days 1,5,9	Control	14	5-13	8.5	—
		1.0	10	8-29	22	158%
		2.0	20	8-43	29	241%
		3.0	19	8-36	10	N
DTIC	Days 1-5	Control	20	5-39	9	—
		0.75	20	3-31	15	66.7%
		1.0	10	5-38	13.5	50%
		1.25	10	7-25	16	77.8%‡
DTIC	Days 1,5,9	Control	10	5-25	8	—
		1.5	10	5-19	13.5	68.8%
		1.75	9	8-15	9	N
Vinblastine	Day 1	Control	10	5-18	12	—
		0.02	10	8-17	8	N
		0.03	10	5-40	12.5	N
		0.04	10	8-22	10	N
Vinblastine	Days 1-5	Control	9	5-14	10	—
		0.005	8	8-26	11	N
		0.006	8	5-26	13.5	N
		0.007	8	5-15	9	N
Vinblastine	Days 1,3,5	Control	9	5-14	10	—
		0.006	7	8-22	15	50%
		0.008	9	7-26	12	N
		0.01	9	5-19	10	N
Vincristine	Days 1-5	Control	10	5-25	8	—
		0.0005	9	8-10	8	N
		0.001	10	5-11	8	N
		0.002	10	5-9	8	N
Vincristine	Days 1,3,5	Control	10	5-19	12	—
		0.004	10	5-12	10	N
		0.005	10	5-10	8	N
		0.006	10	5-9	8	N

*Control mice received 0.2 ml of saline ip.
†%ILS = percent increase in lifespan of treated animals over that of controls (median survival time); N = ILS < 50%.
‡ Significant difference in lifespan between treatment group and control identified at $P < 0.05$.

active drugs selected by the C1300 murine neuroblastoma system were also selected by the "screening" tumors. However, unlike the usual animal screens for antitumor agents, this study attempts to study specific activity. If the C1300 model is a reliable screen, it should lead to improved therapy and prognosis for children with metastatic neuroblastoma. These experiments suggest that children with metastatic neuroblastoma may respond to cyclophosphamide, isophosphamide, and BCNU, and, although less active against the model, that DTIC, adriamycin, ICRF-159, guanazole, and the vinca alkaloids may also be effective. The results suggest that agents selected by the C1300 model should be given adequate clinical trials.

Clinical studies reveal that cyclophosphamide is the agent of choice in the treatment of childhood disease (12). BCNU has not received an adequate trial in newly diagnosed children, although the nitrosoureas are active in human tumors. Isophosphamide has been used in a limited trial by members of the Acute Leukemia Group B, and measurable tumor regression was observed in a small number of children who had not received prior therapy with cyclophosphamide.[6] The borderline activity demonstrated in the model for the vinca alkaloids, adriamycin, and DTIC correlates with clinical experience. While these agents are active in children with stage IV disease, they do not appear to have the clinical efficacy of cyclophosphamide. ICRF-159, cytosine arabinoside, and guanazole have not received widespread clinical trial. The experiments with the animal model suggest that further clinical studies are warranted with DTIC, ICRF-159, adriamycin, gua-

[6]Truman JT. Personal communication.

TABLE 3.—Response of C1300 neuroblastoma to chemotherapy*

Group I	Group II	Group III
Cyclophosphamide	DTIC	5-FU
Isophosphamide	Adriamycin	Vincristine
BCNU	Guanazole	Bleomycin
Cytosine arabinoside†	Vinblastine	Acronycine
	ICRF-159	Actinomycin D†
		Methotrexate†

*Group I drugs consist of those agents which cause greater than 50% reduction in tumor volume and significantly increase animal lifespan 50% or more. Group II drugs do not cause tumor regression but do prolong animal lifespan 50% or more. Group III drugs do not cause tumor regression and fail to prolong animal lifespan 50% or more.

†Results for cytosine arabinoside, actinomycin D, and methotrexate have been previously reported (ref 10).

nazole, and the vinca alkaloids either alone or in combination. Clinical studies by the Children's Cancer Study Group appear to support the value of the model since DTIC is active as a single agent in the human tumor, and combination therapy with DTIC, vincristine, and cyclophosphamide is extremely efficacious in children with widespread disease (15).

LIST OF COMPOUNDS

Acronycine: NSC-403169; CAS reg. No. 7008-42-6; 7*H*-pyrano-[2,3-c]acridin-7-one, 3,12-dihydro-6-methoxy-3,3,12-trimethyl-

Actinomycin D: NSC-3053

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Bleomycin: NSC-125066

Bretylium tosylate: NSC-62164; CAS reg. No. 61-75-6; ammonium, (o-bromobenzyl)ethyldimethyl—*p*-toluenesulfonate

Butyric acid: NSC-8415; CAS reg. No. 107-92-6; sodium butyrate

Cyanocobalamin: NSC-80365; CAS reg. No. 68-19-9; vitamin B_{12}

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

Guanazole: NSC-1895; CAS reg. No. 503-88-8; 1,2,4-triazolidine, 3,5-diimino-

6-Hydroxydopamine: NSC-233898; 1,2,4-benzenetriol, 5-(2-aminoethyl)-, hydrochloride

ICRF-159: NSC-129943; CAS reg. No. 21416-87-5; 2,6-piperazinedione, 4,4′-propylenedi-, (±)-

Isophosphamide: NSC-109724; CAS reg. No. 3778-73-2; 1,3,2-oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]-tetrahydro-, 2-oxide

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Papaverine: NSC-35443; CAS reg. No. 61-25-6; isoquinoline, 6,7-dimethoxy-1-veratryl-, hydrochloride

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Preliminary Observations on Temperature-Enhanced Drug Uptake by Leukemic Leukocytes In Vitro[1,2]

Jerome B. Block, Phillip A. Harris, and Ann Peale[3,4]

A recent review (1) in this journal examined the potential role of hyperthermia in causing tumor regression in vivo and prompted a series of provocative letters on the topic (2,3). Other reports (4-7) have extended previous observations on the interactions of X-irradiation, heat, and anticancer drugs in killing tumor cells in vitro. In general, these latter studies have demonstrated heat effects most clearly at temperatures above 41°C and have not related anticancer drug cellular uptake to an induced tumor temperature change. This report presents preliminary data which indicate that anticancer drug uptake may be enhanced in vitro at only moderate elevations in temperature and that these temperatures are not associated with gross changes of in vitro tumor growth kinetics.

METHODS

L1210 Leukemia Culture

L1210 leukemia[5] was grown in triplicate stationary cultures in stoppered flat sterile plastic flasks (Falcon and Costar) using 10% fetal calf serum in RPMI 1640 culture media (Associated Biomedical System, Inc., Buffalo, NY) containing L-glutamine, penicillin (100 units/ml), and streptomycin (50 μg/ml) at transplant inoculations of 3×10^5 viable[6] cells/ml. The 37°C growth characteristics are shown in figure 1. Viability was > 98% on Day 4 of culture and then fell to 40%-50% by Day 8. Four-day-old cultures were used for drug uptake studies.

Human Leukemia Leukocyte Collection

Viable fresh leukocyte preparations were obtained by aspiration of white blood cell-rich plasma from sedimented whole heparinized venous blood of patients with leukemia. Cells were adjusted to desired concentrations by suspension in RPMI 1640 media with 10% fetal calf serum. All patients had untreated progressive disease.

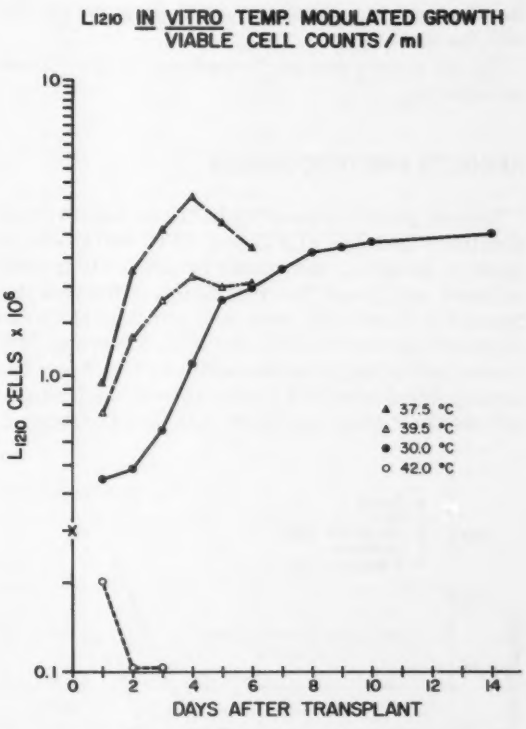

FIGURE 1.—Effect of temperature on growth characteristics of L1210 leukemia in vitro.

Drug Uptake Studies

Leukemic cells were incubated in open agitated tubes at cell concentrations between 10^6 and 10^7 cells/ml of culture media for 2 hours at the noted temperatures at initial drug concentrations of 1-5 μg/ml of adriamycin. Cytocrits were <0.05. At the beginning of each incubation, immediately following drug addition to a completely equilibrated culture, an aliquot of cells was spun down (see below) and analyzed for a "blank" or 0 time drug uptake level. Following full incubation with drug, cells were spun at 1000 rpm for 10 minutes at ambient

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[5]A total of 10^5 cells when inoculated in BDF_1 mice killed within 15 days.

[6]Exclusion of 0.5% trypan blue in saline.

temperature. The supernatant was discarded. When cells were washed in 20-fold volumes of RPMI 1640 media or saline, analytic studies demonstrated loss of drug. Therefore spun cell buttons were directly analyzed for drug uptake. Adriamycin was analyzed spectrofluorometrically as previously described (8). Results were expressed as μg of drug uptake/10^9 cells/incubation.

All cell counts were performed on a Coulter Counter model Z_{BI}.

RESULTS AND DISCUSSION

Typical growth curves for L1210 at selected temperatures between 37.5°C and 42°C are shown in figure 1. In table 1 cell counts for all L1210 growth cultures are given. No significant differences between 37.5°C and 40°C were seen although the trend of growth is lower at 39°C and 40°C. Growth at 23°C is poor and at temperatures above 41°C cultures are usually killed within 24 hours. Adriamycin leukemic cell uptake studies are shown in table 2 and figure 2.

Adriamycin uptake was appreciably greater at 39.5°C than at 37.5°C after 1-2 hours' incubation. These results were not seen in the 0 time incubation assays. Figure 3 demonstrates that these differences are not obliterated after more prolonged incubation.

These preliminary results in human and murine leukemic leukocytes suggest that temperature may have important effects on enhancing drug uptake, especially at temperatures above 37°C. Clinically, elevations to 40°C during chemotherapy are not uncommon and may be achievable at limited patient risk by such techniques as microwave irradiation, heated blood perfusion, or exterior blankets or heating garments. Considerable work is needed to extend the observations in this report to normal tissues, to in vivo studies, and to observations on other drugs. The data suggest a somewhat discontinuous effect of heat on drug uptake which has not been adequately considered previously and which may have clinical relevance. In support of these data a recent report (9) has demonstrated increased adriamycin fluorescence in Chinese hamster cells in vitro at 43°C using a cytofluorographic technique.

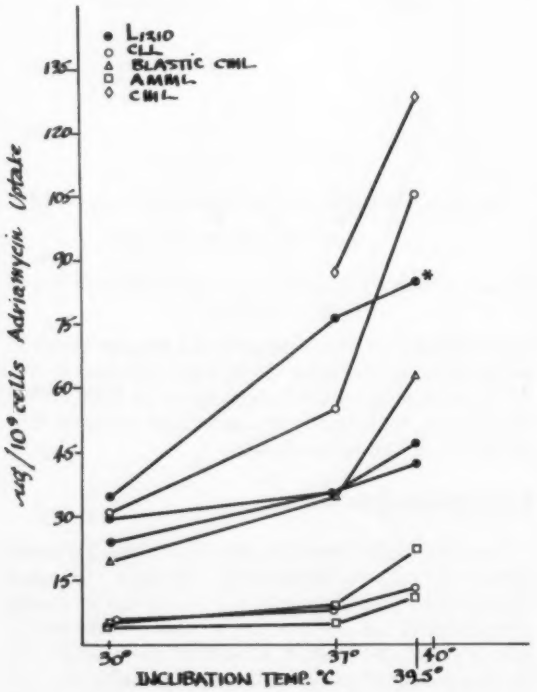

FIGURE 2.—Effects of temperature on adriamycin uptake by leukemic leukocytes during short-term in vitro culture. Adriamycin concentrations in individual experiments varied from 1 to 5 μg/ml. *Experiments with these leukocytes represent cell concentrations after two cell washings.

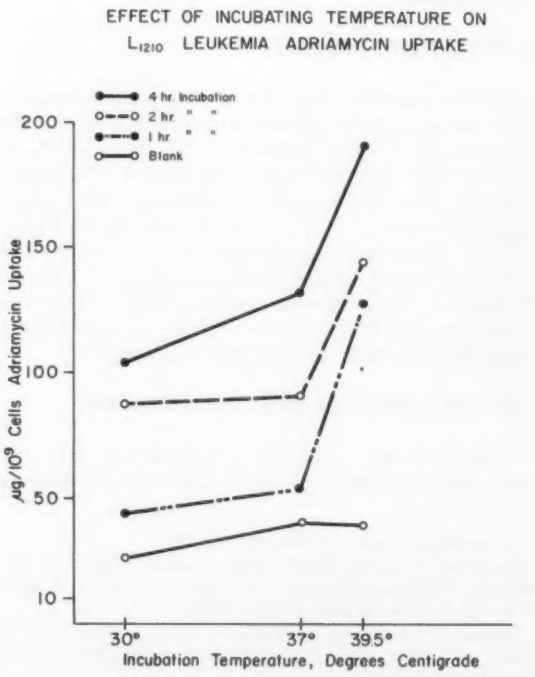

FIGURE 3.—Influence of prolonged incubation on adriamycin uptake at differing temperatures. Adriamycin concentration in the incubating media was 10 μg/ml. The increased drug uptake between 37°C and 39.5°C seen at 1 and 2 hrs of study was maintained through 4 hrs. 0 time (blank) adriamycin uptake did not show this increment.

TABLE 1.—In vitro L1210 leukemia culture

Temperature (C)	No. of cultures studied	Mean ± SD viable cell counts × 10^6/ml on Day— 1	2	3	4	5	7
30°	4	0.44	0.57	0.83	1.23	1.59	1.84
		0.05	0.19	0.32	0.55	0.60	0.60
37.5°	15	0.61	1.64	2.75	3.09	3.42	2.62
		0.24	0.49	0.52	0.80	0.80	0.77
39°-40°	4	0.73	1.50	2.23	2.50	2.83	3.01
		0.21	0.22	0.19	0.15	0.04	0.11
41°-42°	2	0.22	0.08	0.09	—	—	—
		0.02	0.07	0.11			

TABLE 2.—Drug uptake (μg/10^9 cells): effect of temperature in vitro

Length of incubation (hrs)	Disease	Human leukemia: Adriamycin uptake at temperature of— 30°	37°	39.5°	L1210 leukemia: Adriamycin uptake at temperature of— 30°	37°	39.5°
0	Chronic myelocytic leukemia in blastic phase	10.23	10.94	15.26			
2		18.94	33.98	62.61	34.38	76.54	87.71*
0	Chronic myelocytic leukemia		21.04	39.55	22.28	24.0	22.77
2			88.42	128.40	29.92	33.98	46.35
0	Acute myelomonocytic leukemia				15.70	17.00	17.70
1		5.6	9.0	22.3	23.70	33.80	42.53
0	Chronic lymphocytic leukemia	11.9	17.06	12.66			
2		31.43	56.14	105.02			
0	Acute myelomonocytic leukemia	1.96	1.67	2.09			
2		3.61	5.48	9.48			
0	Chronic lymphocytic leukemia	1.39	1.58	1.31			
2		3.83	8.80	12.90			

*Washed cell study.

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Dibromodulcitol (NSC-104800) Compared With Cyclophosphamide (NSC-26271) as Remission Maintenance Therapy in Previously Treated Children With Acute Lymphoblastic Leukemia or Acute Undifferentiated Leukemia: Possible Effectiveness in Reducing the Incidence of Central Nervous System Leukemia[1,2]

A. L. Sitarz, V. Albo, N. Movassaghi, M. Karon, D. Hammond, J. Weiner, and A. Reed[3,4]

SUMMARY

Dibromodulcitol and cyclophosphamide are both alkylating agents. In this study, these two drugs were compared for their effectiveness as remission maintenance therapy for childhood acute lymphoblastic leukemia or acute undifferentiated leukemia. Toxic effects were similar in both groups of patients although cystitis did not occur with the dibromodulcitol treatment. The duration of remission was slightly shorter for dibromodulcitol than for cyclophosphamide ($P = 0.04$). There was, however, a lower incidence of CNS leukemia in the patients treated with dibromodulcitol, which did not seem to be related to a basic difference in the patient groups.

[Cancer Chemother Rep 59:989-994, 1975]

Cyclophosphamide has been previously shown (study No. CCG-805)[5] to produce a median remission maintenance time of 109 days at an actual remission maintenance dose of 2.6 mg/kg/day in children with acute lymphoblastic leukemia (ALL) or acute undifferentiated leukemia (AUL). This result was similar to that reported by Pierce et al (1), and was little better than the results reported in children who had had no maintenance chemotherapy after a remission induced with vincristine (2).

Chemically, dibromodulcitol is a substituted hexitol (fig 1) and functions as an alkylating agent. It has been shown to produce profound myelosuppression in animals and in humans (3-5), and in a prior study conducted by the Children's Cancer Study Group (CCSG) (study No. CCG-920; M. Karon, unpublished data) dibromodulcitol induced one remission in a group of 20 children with ALL. The disease in 19 of the 20 children was already resistant to cyclophosphamide but the degree of cross-reactivity

[1]Received Jan 17, 1975; revised July 7, 1975; accepted July 23, 1975.

[2]Dibromodulcitol: CAS reg. No. 10318-26-0; galactitol, 1,6-dibromo-1,6-dideoxy-.

Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

[3]This study (No. CCG-805-A) was conducted by the Children's Cancer Study Group. The following institutions participated in the study: Children's Hospital of Washington, DC (*S. Leikin* and *N. Movassaghi*, CA-03888); Children's Hospital of Los Angeles, Calif (*M. Karon* [deceased], *G. Higgins*, *C. Hyman*, *J. Ortega*, *N. Shore*, and *K. Williams*, CA-02649); University of Southern California, Los Angeles (*D. Hammond* and *J. Weiner*, CA-13539); Babies Hospital, New York, NY (*J. Wolff* and *A. Sitarz*, CA-03526); Children's Hospital of Pittsburgh, Pa (*V. Albo*, *W. Prin*, and *S. Orlando*, CA-07439); Children's Hospital of Columbus, Ohio (*W. Newton* and *I. Ertel*, CA-03750); Children's Orthopedic Hospital, Seattle, Wash (*R. Chard*, *J. Hartmann*, and *I. Bernstein*, CA-10382); University of Wisconsin, Madison (*P. Joo*, CA-05436); University of Minnesota, Minneapolis (*W. Krivit* and *M. Nesbit*, CA-07306); Children's Memorial Hospital, Chicago, Ill (*W. Borges*, *A. Schwartz*, *M. Pierce*, *J. Nicklas*, and *E. Hackl*, CA-07431); University of Utah Medical Center, Salt Lake City (*E. Lahey* and *J. Wilson*, CA-10198); University of Rochester, NY (*M. Klemperer*, CA-11174); Milwaukee Children's Hospital, Wis (*L. Thatcher* and *S. McCreadie*, CA-11075); Children's Hospital of Philadelphia, Pa (*A. Evans*, *M. Donaldson*, and *H. Carim*, CA-11796); Cornell Medical Center, New York, NY (*D. Miller*, CA-14557); Indiana University, Indianapolis (*R. Baehner* and *R. Wheetman*, CA-13809); New Jersey College of Medicine, Newark (*L. Vitale*, CA-12637); Harbor General Hospital, Torrance, Calif (*J. Finklestein*, *L. Sieger*, *L. Greenberg*, and *V. Medrano*, CA-14560); University of British Columbia, Vancouver, Canada (*M. Teasdale*); and Princess Margaret Hospital, Toronto, Canada (*M. Sonley*, Ontario Cancer Treatment and Research Foundation grant 41-17).

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[4]*Reprint requests to:* Anneliese L. Sitarz, MD, Department of Pediatrics, College of Physicians and Surgeons, Columbia University, 620 West 168th St, New York, NY 10032.

[5]Albo V, Movassaghi N, Sitarz A, et al. Cyclophosphamide therapy for maintenance following a second remission of acute lymphoblastic leukemia of childhood: a comparative clinical trial (standard dose vs intermittent high dose vs cyclophosphamide plus cytosine arabinoside). In preparation.

was not known. The one child in whom an M_1 (6) marrow developed had not received cyclophosphamide previously.

Hemorrhagic cystitis, which has been repeatedly seen with cyclophosphamide therapy, has not been reported with dibromodulcitol. The present study (No. CCG-805-A) was done to determine whether dibromodulcitol might have superior remission maintenance potential but less toxicity than cyclophosphamide.

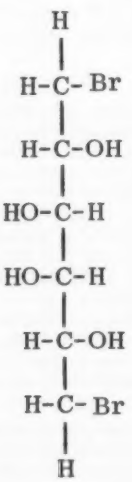
MATERIALS AND METHODS

All children with ALL, AUL, or stem cell leukemia were eligible for this study if they *(a)* had previously received and their disease was resistant to 6-mercaptopurine and methotrexate, *(b)* had received only one previous course of steroids, and *(c)* had received no prior cyclophosphamide. As in study No. CCG-805, children were acceptable who had received additive doses of mechlorethamine (one injection every 8 weeks) as a part of study Nos. CCG-802 and 803 (7). The bone marrow was required to be M_3 (6) at the start of this study.

Induction treatment consisted of prednisone (2 mg/kg/day for 28 days, tapering to zero by 42 days) and vincristine (0.075 mg/kg/week for six doses) (fig 2).[6]

[6]Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna 1,4-diene-3,11,20-trione, 17, 21-dihydroxy-.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).



1, 6-dibromo-1, 6-dideoxy-galactitol

FIGURE 1.—Dibromodulcitol.

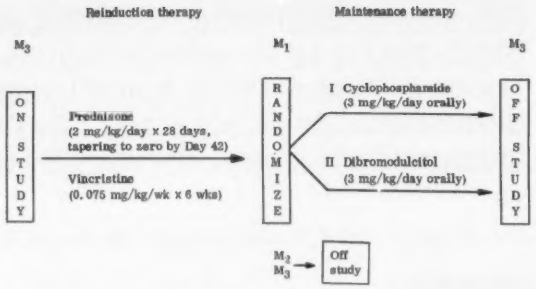


FIGURE 2.—Schematic diagram of reinduction and maintenance protocol.

Those patients who achieved an M_1 marrow by Day 42 of the induction treatment were randomized to receive 3 mg/kg/day of either cyclophosphamide or dibromodulcitol orally for maintenance therapy. If the marrow was M_2 or M_3, the child was taken out of the study.

All patients were followed with a complete blood cell count and a physical examination every 4 weeks, and a bone marrow examination every 8 weeks during maintenance therapy. Urinalysis was performed monthly or when symptoms occurred. Patients were removed from the study if an M_3 marrow or severe drug toxicity occurred.

As the study progressed, the patient accrual rate was less than had been anticipated. Since more was known about standard-dose cyclophosphamide than about dibromodulcitol, the protocol was modified in October 1972 to admit all eligible patients to the dibromodulcitol arm of the maintenance phase for the last 8 months of the study.

Nonparametric methods were used in the analysis of these data and in tests of significance (8).

RESULTS

Between March 1971 and June 1973, 50 patients were entered into the induction phase of this study. Of these 50 patients, 45 achieved M_1 marrows (90%). Five patients were lost from the study after achieving M_1 marrows. In four of these patients there was a failure to follow the protocol in the maintenance phase (the drug was given intermittently), and the fifth patient died of sepsis before the start of maintenance therapy.

Most of the patients had participated in study No. CCG-803, and all but four had had no prior vincristine. Two of the four patients who had received vincristine had only one or two doses, and the two other patients had achieved a prior complete remission with the drug. Some of the patients had had previous pulse doses of mechlorethamine as part of the CCG-803 treatment regimen.

Of 40 patients who were eligible for randomization in the maintenance phase, 17 were entered into the cyclophosphamide regimen and 23 into the dibromodulcitol regimen. All but one of these patients has since relapsed. The exception is a child who is still receiving cyclophosphamide daily and has been on maintenance therapy for 1168 days.

Remission duration was slightly longer for patients receiving cyclophosphamide than for those receiving dibromodulcitol. The median time on maintenance therapy was 134 days for patients receiving cyclophosphamide and 63 days for those receiving dibromodulcitol, which shows a slight statistical difference ($P = 0.04$).

Although the numbers are relatively small, the two groups of patients were evaluated statistically in retrospect for various factors which might have had prognostic significance. These factors included the white blood cell count (wbc) at the time of diagnosis, the administration of pulse doses of mechlorethamine during prior treatment, the patient's age at diagnosis, and the duration of previous therapy. No significant differences were observed between the two groups, including whether or not mechlorethamine had been previously received (table 1).

No significant differences were observed in the median dose of maintenance drug received or in the hematologic toxicity encountered between the two treatment groups (table 2). The median time to nadir for wbc and platelet count tended to favor cyclophosphamide. The median nadir of values for wbc was higher, however, in patients receiving dibromodulcitol.

Cystitis occurred in two patients who received cyclophosphamide and in none who received dibromodulcitol.

Extramedullary Leukemia

Central nervous system (CNS) leukemia developed in nine of the 17 patients receiving cyclophosphamide and in only one of the 23 patients receiving dibromodulcitol ($P = 0.001$) (9) (table 3). This interesting finding led to a retrospective evaluation of possible factors that could account for this. The two groups appeared comparable in that CNS leukemia had occurred during prior treatment phases in ten of the patients in the cyclophosphamide group and in seven of those receiving dibromodulcitol. The median time interval between the occurrence of previous CNS leukemia and the start of this study was 103 days (dibromodulcitol group) and 104 days (cyclophosphamide group). In addition, the number of previous CNS episodes averaged two for each group (range 1-4), and the treatment received was similar. Nearly all of the patients received methotrexate[7] intrathecally, and eight patients also received cranial radiation (all but one in the cyclophosphamide group). In all patients lumbar punctures were performed only when symptoms referable to the CNS occurred. None of the patients had had prophylactic CNS treatment. Of those patients with a history of prior CNS involvement, six of the ten in the cyclophosphamide group had a recurrence during this study, whereas this occurred in only one of the seven patients in the

[7]Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

TABLE 1.—Time on maintenance therapy comparing cyclophosphamide and dibromodulcitol within prognostic subgroups

	Treatment (3 mg/kg/day, orally)			
	Cyclophosphamide		Dibromodulcitol	
Prognostic subgroups	No. of patients	Median maintenance time (days)	No. of patients	Median maintenance time (days)
Wbc				
<20,000 cells/mm^3	16	138	19	84
≥20,000 cells/mm^3	1	112	4	61
Mechlorethamine treatment				
Previous	7	151	8	54
No previous	10	126	15	106
Age of patient				
≤3 yrs	2	103	6	46
>3 yrs	15	143	17	106
Prior treatment				
<2 yrs	8	130	12	55
≥2 yrs	9	134	11	119

TABLE 2.—Median and range for the hematologic toxicity parameters analyzed

	Treatment (3 mg/kg/day, orally)			
	Cyclophosphamide		Dibromodulcitol	
Parameter	Median	Range	Median	Range
End of induction phase—				
Hemoglobin level (g/100 ml)	11.7	6-14	12.0	7.1-14.7
Wbc (cells × 10^3/mm^3)	7.9	4.5-10.1	5.7	2.0-17.8
Platelet count (platelets × 10^3/mm^3)	200.0	67.0-389.0	213.0	40.0-560.0
Nadir for—				
Hemoglobin level (g/100 ml)	10.8	8.3-12.8	10.1	7.1-13.6
Wbc (cells × 10^3/mm^3)	1.9	1.1-5.0	2.6	0.5-6.9
% granulocytes at wbc nadir	63.6	36.0-86.0	44.0	12.0-84.0
Platelet count (platelets × 10^3/mm^3)	98.5	35.0-225.0	71.3	10.0-225.0
Days to nadir for—				
Hemoglobin	41.5	14.0-166.0	53.0	4.0-167.0
Wbc	63.5	14.0-210.0	53.0	14.0-166.0
Platelet count	70.5	15.0-177.0	52.0	13.0-126.0
Days on maintenance therapy	134.0	70.0-1168.0	63.0	14.0-224.0
Days of previous disease	774.0	371.0-1686.0	716.0	323.0-1609.0
% of protocol dose received	79.5	37.0-101.0	85.0	34.0-153.0

TABLE 3.—Time on maintenance therapy comparing cyclophosphamide and dibromodulcitol within subgroups defined by the incidence of CNS disease before and during this study

	Treatment (3 mg/kg/day, orally)			
	Cyclophosphamide		Dibromodulcitol	
Subgroups	No. of patients	Median maintenance time (days)	No. of patients	Median maintenance time (days)
No CNS disease during this study				
With previous CNS disease	4	123	6	53
Without previous CNS disease	4	132	16	86
CNS disease during this study				
With previous CNS disease	6	115	1	69
Without previous CNS disease	3	164	0	—

dibromodulcitol group. Although this indicates a marked difference between the two groups with respect to recurrence of CNS leukemia, the difference was not statistically significant because of the small sample size. While the median times on maintenance therapy for both groups of patients showed only a slight statistical difference, there did seem to be some relationship between the length of time on

maintenance therapy and the development of CNS leukemia. In eight of the nine cyclophosphamide-treated patients who had CNS leukemia, CNS symptoms developed 3 months after the drug was started. In the one patient receiving dibromodulcitol who had CNS leukemia, the symptoms developed on Day 23 of maintenance therapy. To determine whether the length of time in the study was a significant factor, the occurrence of CNS leukemia was examined in those patients whose marrow relapse came early. The patient groups were again comparable in that CNS leukemia developed in only one patient in each group within 3 months of leaving this study.

The percent of the protocol-specified dose of drug received was comparable in both groups of patients, although the one patient receiving dibromodulcitol in whom CNS symptoms developed had received the drug for only 23 days. There did not seem to be any correlation between previous treatment with mechlorethamine and the occurrence of CNS leukemia.

Testicular involvement occurred in one patient who was receiving cyclophosphamide.

No unusual infections were reported.

DISCUSSION

In this group of previously treated children with ALL or AUL, the length of remission maintenance therapy for the dibromodulcitol group was slightly shorter than that for the cyclophosphamide group. The median number of days on maintenance therapy with cyclophosphamide in this study was somewhat longer than previously reported (1).[8] It should be noted that the median duration of disease for the patients who came to this study from study No. CCG-803 was 774 days (range 371-1687 days). This median is significantly longer than that reported for patients who had been entered into the preceding study (No. CCG-805) (median 280 days; range 82-694 days) and may reflect the fact that the current patient group had better responses to treatment. The median doses of cyclophosphamide and dibromodulcitol administered to the two groups and the hematologic toxicity encountered were similar. Cystitis occurred in two patients receiving cyclophosphamide and in none receiving dibromodulcitol. The lower incidence of CNS leukemia in the patients receiving dibromodulcitol was a provocative finding.

Belej et al (5,10) have shown that dibromodulcitol given orally is rapidly absorbed from the gastrointestinal tract and achieves maximum blood levels within 1 hour. These investigators also showed that dibromodulcitol compounds entered spinal, pleural, and ascitic fluids, reaching a concentration equivalent to one half the maximum serum level approximately 5 hours after administration. Studies done in mice showed that the drug also rapidly penetrates most tissues and equilibrates with plasma levels within 2 hours.

Although the identity of the compound present in the cerebrospinal fluid has not been characterized, studies with radioactive labeling have shown it to be either unchanged drug or its monobromo derivative (5).

CONCLUSION

This study was designed to determine whether dibromodulcitol would be a better drug than cyclophosphamide for remission maintenance therapy for childhood ALL or AUL. In fact, the duration of remission was slightly shorter for dibromodulcitol than for cyclophosphamide, a difference that was of borderline significance ($P = 0.04$). Toxicity in the two groups was similar except that cystitis did not occur in the patients receiving dibromodulcitol. An unexpected and provocative finding was a lower incidence of CNS leukemia in the patients receiving dibromodulcitol which did not seem to be related to a basic difference in the patient groups.

In view of the small number of patients, the clinical significance of these CNS data needs to be further evaluated.

Since dibromodulcitol is known to cross the blood-brain barrier, it would seem important to incorporate this drug into treatment regimens to evaluate further its effectiveness in preventing CNS involvement in patients with leukemia.

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[8] See footnote 5.

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Phase II Study of *Cis*-dichlorodiammineplatinum(II) (NSC-119875) in Combination With Cyclophosphamide (NSC-26271) in the Treatment of Human Malignancies[1,2,3]

Ira J. Piel and Charles P. Perlia[4,5]

SUMMARY

The effectiveness of *cis*-dichlorodiammineplatinum(II) in the treatment of human malignancies is evaluated. The first stage of our investigation consisted of a phase I study to determine toxicity. In the second stage attempts were made to reduce toxicity by varying the modes of administration, and the third stage comprised studies of combination chemotherapy including *cis*-dichlorodiammineplatinum(II). A total of 74 patients have been treated, 20 of whom received the combination of *cis*-dichlorodiammineplatinum(II) and cyclophosphamide. Major toxic effects included vomiting, mild leukopenia and thrombocytopenia, decreased creatinine clearance, audiologic toxic effects, hyperuricemia, and nephrotoxicity. Measurable regression of tumors was seen in 18 of the 74 patients, and ten of the 18 patients who responded had been given the combination of *cis*-dichlorodiammineplatinum(II) and cyclophosphamide.

[Cancer Chemother Rep 59:995-999, 1975]

Clinical studies at the Rush-Presbyterian-St. Luke's Medical Center using *cis*-dichlorodiammineplatinum(II) have been performed in three stages. The original stage was essentially a phase I drug study, part of which was reported in May 1972 (1).[6] Some experience with the acute and chronic toxic characteristics of *cis*-dichlorodiammineplatinum(II) was obtained as well as information on the therapeutic efficacies of different dosage levels of the drug. A total of 42 patients were studied in this stage.

In the second stage, attempts were made to reduce the toxic effects by different modes of administration. It was found that *cis*-dichlorodiammineplatinum(II) given by intravenous (iv) drip at a rate of approximately 1 mg/minute in a dilution of 1 mg/ml to well-hydrated patients and followed by continuous iv hydration for a period of at least 24 hours resulted in diminished hematologic and renal toxicity. The problem of hyperuricemia was eliminated by giving allopurinol 1 day prior to treatment and 5-10 days after treatment.

In the third stage, *cis*-dichlorodiammineplatinum(II) was studied in combination with other antitumor agents. Pilot studies with 5-fluorouracil or cyclophosphamide were performed with 19 patients. This paper reports primarily on studies using a combination of cyclophosphamide and *cis*-dichlorodiammineplatinum(II).

MATERIALS AND METHODS

Patients with histologically proven malignant tumors who were not eligible for protocol studies of higher priority were selected. All patients had easily measurable lesions not treated previously with radiation therapy. Normal leukocyte and platelet counts and a creatinine clearance greater than 70 ml/minute were required for all patients. They were hospitalized and received 100-200 ml of fluid per hour iv for a minimum of 12 hours prior to receiving chemotherapy. Allopurinol was started at the time of admission to the hospital. Cyclophosphamide at a dose of 1000 mg/m^2 was administered by iv bolus and was followed by *cis*-dichlorodiammineplatinum(II) at a dose of 75 mg/m^2 dissolved in 1 ml of sterile water/mg of drug and administered at a rate of 1 ml/

[1]Received Feb 17, 1975; revised July 22, 1975; accepted July 23, 1975.

[2]Presented in part at the 2nd International Symposium, Oxford, England, Apr 16-18, 1973.

[3]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

[4]Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill.

[5]*Reprint requests to:* Ira J. Piel, MD, Section of Medical Oncology, Department of Medicine, Illinois Masonic Medical Center, 836 Wellington Ave, Chicago, Ill 60657.

[6]Presented at the Eighth Annual Scientific Meeting, American Society of Clinical Oncology, Inc., Boston, Mass, May 3, 1972.

minute. Both drugs were repeated at 3-week intervals providing renal and hematologic function had returned to normal; otherwise, dosage was modified or administration postponed.

RESULTS

Seventeen of 20 patients treated with this combination regimen are evaluable (table 1). Table 2 shows a breakdown of the evaluable patients by diagnosis. Ten of these patients responded; however, only two patients treated with the combination had greater than 50% reduction in the size of their measurable lesions. The remaining eight responders showed a 25%-50% reduction in the size of their lesions. The duration of response was brief in all patients. The unknown primary group included one patient (the nonresponder) with an undifferentiated adenocarcinoma in a left supraclavicular lymph node, a second with a small cell carcinoma thought to originate in either the thyroid or the trachea, and another (patient No. 1) with a small cell undifferentiated carcinoma with extensive pulmonary metastases and bilateral cervical and axillary nodes.

TABLE 1.—Patients receiving *cis*-dichlorodiammineplatinum(II) plus cyclophosphamide

Patient No.	No. of doses received*	Response
1	XXXX	Yes
3	XXXX	Yes
4	XXX	
5	XXX	Yes
6	XXX	Yes
7	XXX	
8	XXX	Yes
2	XY	Yes
9	XX	
10	XX	
11	XX	Yes
12	XX	Yes
13	XX	Yes
14	XX	Yes
15	X	
16	X	
17	X	
18	X	
19	X	
20	X	

*X = dose of *cis*-dichlorodiammineplatinum(II) plus cyclophosphamide. Y = dose of *cis*-dichlorodiammineplatinum (II) alone.

Case Reports

Patient No. 1.—In November 1972 a 34-year-old woman noted pain in both arms, and in December she had an abnormal chest roentgenogram. On January 2, 1973, a bronchoscopy and scalene node biopsy were performed. Thoracotomy and a lung biopsy done several days later revealed small cell anaplastic carcinoma but failed to show the primary site. A laparoscopy, performed because the histologic studies suggested granulosa cell tumor, was negative. Ten days after her first course of therapy with *cis*-dichlorodiammineplatinum(II) plus cyclophosphamide, she had an impressive clearing of pulmonary nodules in the right lung and in the upper part of the left lung, with shrinkage of the remaining nodules in the lower left lung fields and disappearance of previously palpable nodes. She received full doses of cyclophosphamide and *cis*-dichlorodiammineplatinum(II) every 3 weeks from January 29, 1973, to April 1973, and showed continued but less dramatic shrinkage of her remaining pulmonary lesions. She had only moderate gastrointestinal and hematologic toxic effects from the combination and they completely disappeared before the next course of therapy was started. She had no audiologic toxic effects but her creatinine clearance decreased progressively, although remaining within normal limits. She died in July 1973 and an autopsy revealed a probable primary tumor in the right psoas muscle which was thought to be embryonal rhabdomyosarcoma.

Patient No. 2.—In September 1971 a 37-year-old man had orchiectomy and retroperitoneal node dissection for embryonal cell carcinoma. Subsequent therapy included radiation therapy to mediastinal, abdominal, and inguinal lymph nodes. In January 1972 he was given methotrexate and in April pulmonary metastases were observed. After June of 1972, he received methotrexate, cyclophosphamide, actinomycin D, and 5-fluorouracil in various combinations. Prior to his first course of therapy, he had severe dyspnea and cough and his chest roentgenogram showed large masses in both lung fields. Twelve days after his first dose of combination chemotherapy, dyspnea and cough had significantly improved and the pulmonary lesions had decreased markedly in size. Unfortunately, even after a 4-week waiting period, his leukocyte count remained below 3000 cells/mm^3. Because his renal function was adequate, *cis*-dichlorodiammineplatinum(II) was administered alone without waiting for hematologic recovery. Subsequently, his chest roentgenogram showed regrowth of several of his pulmonary metastases.

TOXIC EFFECTS

The most common adverse side effect was nausea and vomiting, with only an occasional patient avoiding this reaction after the first exposure to the drug.

TABLE 2.—Response to *cis*-dichlorodiammineplatinum (II) plus cyclophosphamide

Tumor	No. of—		
	Patients treated	Evaluable patients	Responders
Sarcoma	6	6	4
Stomach/colon	3	3	0
Lung	3	0	0
Head/neck	2	2	1
Testis	1	1	1
Hodgkin's disease	1	1	1
Ameloblastoma	1	1	1
Unknown primary	3	3	2

All patients receiving more than 50 mg/m^2, even in small divided doses, experienced nausea, vomiting, and anorexia starting 3-6 hours after injection and lasting as long as 48 hours. Pretreatment with antiemetics, even at very high doses (eg, 20-30 mg of prochlorperazine or equivalent doses of similar agents), had only partial effects upon these symptoms.

Hematologic toxicity occurred in only 25% of the patients. It was most frequently manifested as a mild leukopenia (3000-4000 cells/mm^3) occurring 2-3 weeks after administration of the drug and returning to normal spontaneously. Thrombocytopenia was seen only sporadically and was never associated with bleeding. As previously reported (1), one third of the patients who had received several courses of *cis*-dichlorodiammineplatinum(II) had significant anemia (decrease in hemoglobin level of 2 g/100 ml). The onset of this anemia was often delayed, usually occurring 3-5 weeks after the start of therapy.

Because of early reports of audiologic toxic effects, serial audiograms were obtained on 25 patients. Forty percent of the patients had a decrease of more than 20 decibels in the perception threshold of an 8-kHz tone and 5% experienced a 20-decibel decrease in perception of a 4-kHz tone in at least one ear. Audiograms were not repeated in any of the patients at late enough dates to determine whether this effect was reversible. Several patients have complained of tinnitus and each of these patients has manifested significant high-tone threshold depression. Significant decrease in discriminatory ability or in speech reception threshold was not observed in any of the patients (2).

Hyperuricemia occurring within 7 days of the administration of *cis*-dichlorodiammineplatinum(II) was observed in early studies. Because of this, therapy with allopurinol was started 1 day before the administration of *cis*-dichlorodiammineplatinum(II) and was continued for 5-10 days thereafter. No significant hyperuricemia was seen in patients given allopurinol.

The major life-threatening toxic effect of *cis*-dichlorodiammineplatinum(II) has been nephrotoxicity. Elevation of the blood urea nitrogen (BUN) or creatinine level is related to the size of each dose, the total dose of *cis*-dichlorodiammineplatinum(II) administered, the time allowed for recovery from previous doses, and the state of hydration of the patient. In patients with normal renal function receiving doses of 75 mg/m^2 or less, these abnormalities were mostly transient and only moderate after a first course. On the other hand they became increasingly more severe and longer lasting in patients receiving *cis*-dichlorodiammineplatinum(II) for a second or third time, even if the serum BUN or creatinine levels had returned to normal. Creatinine clearance provided a better indication of renal function than did BUN or serum creatinine. Significant nephrotoxicity (decrease in creatinine clearance to less than 50 mg/minute) occurred only in those patients receiving doses greater than 50 mg/m^2 of *cis*-dichlorodiammineplatinum(II). Twenty-five percent of the patients in the early studies had BUN elevations greater than 25 mg/100 ml after the second course of therapy. A renal biopsy obtained in a patient 2 weeks after a dose of 100 mg/m^2 of *cis*-dichlorodiammineplatinum(II) showed a pattern of acute tubular necrosis in the proximal tubules. Neither glomerular damage nor significant interstitial infiltration was seen. The patient's BUN had risen to 60 mg/100 ml (creatinine 2.1 mg/100 ml, uric acid 15.3 mg/100 ml) by Day 4 but had returned to 14 mg/100 ml (creatinine 1.4 mg/100 ml, uric acid 3.6 mg/100 ml) by the time of biopsy. The patient died several months later, at which time autopsy revealed completely normal renal architecture. With the administration of iv fluid both before and for a minimum of 24 hours after administration of *cis*-dichlorodiammineplatinum(II), nephrotoxicity has

been minimal; this was not the case if the patient somehow escaped the iv hydration. Two patients have required acute hemodialysis but fortunately, no deaths attributable to drug toxicity have occurred.

The toxic effects observed in those patients receiving various combinations are very similar both qualitatively and quantitatively to those noted in patients receiving *cis*-dichlorodiammineplatinum(II) alone, with the toxicity of the second drug superimposed. In other words, in addition to the toxic effects discussed above for *cis*-dichlorodiammineplatinum(II) alone, patients receiving cyclophosphamide and *cis*-dichlorodiammineplatinum(II) tend to have leukopenia occurring between the tenth and the 14th day, and alopecia usually starts approximately 3 weeks after treatment.

DISCUSSION

Since October 1971 *cis*-dichlorodiammineplatinum (II) has been administered either alone or in combination to 74 patients as shown in table 3. These patients include the 42 patients who received *cis*-dichlorodiammineplatinum(II) alone and the 20 patients who received the high-dose combination of cyclophosphamide and *cis*-dichlorodiammineplatinum(II) as described previously. Also included are a pilot series of 14 patients who received *cis*-dichlorodiammineplatinum(II) in combination with 5-fluorouracil at various dosage levels, and five patients who received cyclophosphamide plus *cis*-dichlorodiammineplatinum(II) at doses other than 1000 and 75 mg/m^2 respectively. Some patients are included in more than one treatment group. In terms of response, patients with measurable disease were considered evaluable if they received a total dose of at least 75 mg/m^2 of *cis*-dichlorodiammineplatinum(II) and if they were observed for a minimum of 3 weeks after the last dose of chemotherapy.

Patients listed in the response column showed a 25% or greater response to any form of *cis*-dichlorodiammineplatinum(II) therapy. The responders listed in the last line under "other" include one patient with ameloblastoma and one with squamous cell carcinoma of the pharynx. Only three patients have had a greater than 50% response. The others had a 25%-50% decrease in measurable disease. It must be emphasized that these regressions are short-lived and are achieved only at the expense of significant discomfort and danger to the patient. The possibility of long-term improvement in the length and quality of survival cannot be determined at this time.

In summary, in the past 1½ years, *cis*-dichlorodiammineplatinum(II) has been administered to 74 patients at various dose levels and in various combinations. We have now discovered a dosage and means of administration which seem tolerable at least in the short term. However, it remains to be shown that such a regimen can be repeated without undue cumulative toxicity.

TABLE 3.—Patients treated with *cis*-dichlorodiammineplatinum (II) alone or in combination

Tumor	No. of—		
	Patients treated	Evaluable patients	Responders
Sarcoma	11	10	5
Lung	8	2	0
Colon	8	6	0
Breast	5	5	2
Hodgkin's disease	5	3	2
Lymphoma	3	1	0
Testis	4	3	2
Stomach	4	3	2
Kidney	3	3	0
Melanoma	3	2	0
Unknown primary	7	6	3
Other	13	8	2

LIST OF COMPOUNDS

Actinomycin D: NSC-3053

Allopurinol: NSC-1390; CAS reg. No. 315-30-0; 1*H*-pyrazolo-[3,4-*d*]pyrimidin-4-ol

***Cis*-dichlorodiammineplatinum(II):** NSC-119875; CAS reg. No. 1853-33-9

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

REFERENCES

1. Rossof AH, Slayton RE, and Perlia CP. Preliminary clinical experience with *cis*-diamminedichloroplatinum(II) (NSC-119875, CACP). Cancer 30: 1451-1456, 1972.
2. Piel IJ, Meyer D, Perlia CP, et al. Effects of *cis*-diamminedichloroplatinum (NSC-119875) on hearing function in man. Cancer Chemother Rep 58:871-875, 1974.

Phase I Study of Thalicarpine (NSC-68075), a Plant Alkaloid of Novel Structure[1,2]

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SUMMARY

An initial clinical trial of daily and weekly × 6 intravenous infusions of thalicarpine, a plant alkaloid of novel structure, was carried out in 36 patients. Twenty-eight patients received 33 courses of single-dose administration at doses of 200-1900 mg/m^2. At the maximum tolerable dose of 1400 mg/m^2, toxic effects included arm pain (nine of ten), central nervous system depression (seven of ten), nausea and vomiting (two of ten), hypotension (two of ten), hypertension (two of ten), arrhythmia (premature ventricular contractions) (one of ten), and electrocardiographic changes (mainly T-wave flattening) (five of ten). At the maximum tolerable dose for weekly administration, 1100 mg/m^2/week × 6, arm pain was seen in seven of eight, central nervous system depression in three of eight, hypotension in one of eight, and electrocardiographic changes in three of eight. The recommended dose for phase II trials is 1100 mg/m^2/week by a 2-hour intravenous infusion.

[Cancer Chemother Rep 59:1001-1006, 1975]

Thalicarpine is an aporphine benzylisoquinoline alkaloid originally isolated from the roots of *Thalictrum dasycarpum* Fisch. and Ave-Lall (1), a member of the family *Ranunculaceae*. Its structure is shown in figure 1. It is one of a group of closely related compounds isolated from members of the genus *Thalictrum* which have been shown to have antitumor activity against a monolayer culture of KB cells (2). It is active against the Walker 256 carcinosarcoma, a maximum increase in lifespan of 285% being seen with a dosage of 64 mg/kg/day × 5 (approximately 320 mg/m^2 of body surface area) intraperitoneally (ip).[8]

In animal toxicity studies (3) the acute manifestations were the most important and included convulsions, apnea, hypotension, and cardiac arrest. Chronic toxic effects were renal and hepatic. In the rat, a 3000-mg/m^2 dose of thalicarpine administered as a 2-hour infusion was the LD50 (3). In the rhesus

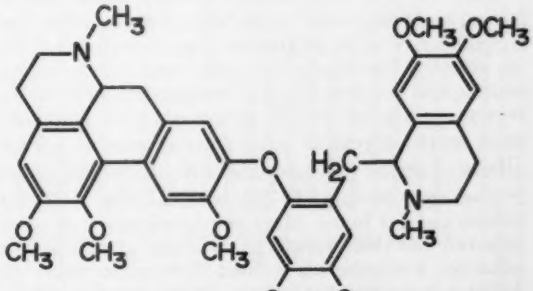

FIGURE 1.—Structure of thalicarpine.

[1]Received Mar 24, 1975; revised June 27, 1975; accepted June 27, 1975.

[2]Thalicarpine: CAS reg. No. 5373-42-2. Supplied by the Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute (NCI).

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[6]The authors wish to thank Dr. F. K. Cantor for performing and interpreting the electroencephalographic (EEG) recordings; Mrs. M. Elis for her help in the conduct of the study; Mr. F. Pelsor and Miss M. J. Bettis for their help in collecting and collating the data; and Mrs. B. Nespoli for her help in preparing the manuscript.

[7]*Reprint requests to:* Patrick J. Creaven, MD, Grace Cancer Drug Center, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

[8]Broder LE, and Carter SK. Clinical brochure: thalicarpine (NSC-68075). Prepared by the NCI, Bethesda, Md, 1971, 39 pp.

monkey, a dose of 2254 mg/m^2 body surface area was a lethal dose by a 1-hour infusion. The high toxic dose was 1080 mg/m^2 and the low toxic dose was 525 mg/m^2 (4). The drug was much more toxic by rapid intravenous (iv) injection than by 1- or 2-hour infusion. Because of its antitumor activity against the Walker 256 carcinosarcoma, because it is the first compound of its class to become available for clinical evaluation, and because of the absence of myelosuppression, we have carried out a phase I study of thalicarpine in patients with advanced solid tumors.

MATERIALS AND METHODS

Patients

Patients with histologically confirmed cancer not amenable to conventional treatment were eligible for inclusion in this study provided their renal function was adequate as determined by a blood urea nitrogen (BUN) level of less than 25 mg/100 ml and a serum creatinine level of less than 1.5 mg/100 ml. Other requirements included a serum bilirubin of less than 1.5 mg/100 ml, a serum glutamic oxaloacetic transaminase (SGOT) value of less than 100 IU, an absence of gross edema, pretreatment white blood cell (wbc) and platelet counts in excess of 5000 cells/mm^3 and 100,000 platelets/mm^3 respectively, a normal electrocardiogram (EKG), and a sufficient interval after previous chemotherapy (2-8 weeks, depending on the preceding treatment) to avoid possible additive toxicity. All patients gave written informed consent before entering this study which was approved by the Human Investigations Subcommittee of the Research and Education Committee of this hospital. A list of the patients studied, their diagnoses, and prior therapy are given in table 1.

The following tests were performed twice weekly during treatment and for at least 3 weeks after the completion of each treatment course: determination of wbc, differential, platelet, and reticulocyte counts, and determination of hematocrit level. BUN, serum creatinine, SGOT, serum alkaline phosphatase, serum bilirubin, total serum proteins, serum albumin, serum calcium, and urinalysis were performed once weekly. A 12-lead EKG was obtained before and 1-2 hours after the termination of each infusion and then weekly for 3 weeks after the last infusion. A rhythm strip (lead II) was run every 15 minutes throughout the course of the infusion. Pulse and blood pressure measurements were made at 15-minute intervals throughout the infusion. Tumors were measured by caliper using the product of the greatest horizontal and vertical diameters. Additional examinations, such as skeletal and chest roentgenograms, were obtained before the study. The chest roentgenograms were repeated every 2 weeks whenever measurable tumors were present; for patients without measurable tumors, roentgenographic examinations were repeated at the end of each study or when clinically indicated.

Drug Administration

Thalicarpine was supplied in ampules containing 100 mg of drug and sufficient phosphoric acid and sodium hydroxide to give a final pH of approximately 6 when reconstituted with water. The contents of the ampules after the addition of 9.8 ml of water were added to 1 liter of 5% dextrose in water (to give a clear to slightly opalescent solution) and administered into an arm vein within 1 hour by constant iv infusion over 2 hours (doses below 1070 mg/m^2 were given in 500 ml over 1 hour). The ampules were stored at -10° C and protected from light. The reconstituted drug was not stored; if not used, it was discarded.

RESULTS

Single-Drug Administration

The starting dose was 200 mg/m^2. Fourteen patients were entered at doses of 200-800 mg/m^2. After an interval of at least 1 month, three of these patients who experienced no toxic effects were reentered and given a higher dose so that in this dose range a total of 17 single-dose infusions were given. Three patients did have some toxic reactions: drowsiness lasting for 12 hours (patient No. 13, 800 mg/m^2); mild hypertension (patient No. 6, 300 mg/m^2); and mild-moderate hypertension (a blood pressure of 120/80 increased to 170/100 in patient No. 4, 400 mg/m^2). Sixteen patients, two of whom (patient Nos. 1 and 12) had received smaller single doses, were each treated with a single dose of 1070-1900 mg/m^2 (tables 1 and 2). Some evidence of drug effect was seen in 15 of these patients. The toxicity observed is shown in table 2. Arm pain was almost universal in this group and twice it was severe enough to necessitate completion of the infusion in the other arm. Drowsiness was common and usually mild; most patients slept during the afternoon after a morning dose. Two cases in which central nervous system (CNS) depression was a clinical problem are discussed in detail below. The EKG changes typically observed are shown in figure 2. Two patients also showed low voltage on the EKG. Levels of serum electrolytes including CA^{++} and K^+ and levels of serum creatine phosphokinase (CPK) showed no change at 4 and 24 hours after

TABLE 1.—Patient information

Patient No.	Age (yrs)	Weight (kg)	Sex	Diagnosis	Prior therapy			Thalicarpine dose	
					Surgery	Radiotherapy	Chemotherapy*	Single infusion (mg/m²)	Weekly infusion (mg/m²/wk)
1	48	72	M	Epidermoid carcinoma, lung		+	VP-16-213, ICRF-159	200, 600, 1070	
2	52	44	M	Adenocarcinoma, colon	+		IFOS, 5FU, VP-16-213	200	
3	54	59	M	Adenocarcinoma, lung		+	VCR, HYDROXY, PROCARB, CYC, MTX, CCNU	200	
4	40	49	M	Cholangiocarcinoma				400	
5	58	60	F	Cholangiocarcinoma				300	
6	51	48	M	Renal cell carcinoma				300	
7	65	68	M	Hepatocellular carcinoma				300	
8	54	73	M	Adenocarcinoma, stomach			IFOS	450	
9	64	38	M	Epidermoid carcinoma, palate		+	5FU, BCNU	450, 600	
10	65	44	M	Epidermoid carcinoma, larynx		+		450, 600	
11	50	65	M	Renal cell carcinoma			ICRF-159, VP-16-213	600	
12	49	39	M	Adenocarcinoma, stomach	+	–		600, 1400	
13	53	44	M	Epidermoid carcinoma, lung		+		800	
14	52	45	M	Epidermoid carcinoma, pharynx		+	VP-16-213	800	
15	66	47	M	Large cell carcinoma, lung				1070	
16	57	56	M	Anaplastic large cell carcinoma, lung		+	ICRF-159, CCNU, VP-16-213	1070	
17	42	62	M	Anaplastic large cell carcinoma, lung	–	–	CYC, MTX	1070	
18	40	40	F	Adenocarcinoma, stomach		+	5FU	1400	800 × 5
19	26	44	M	Epidermoid carcinoma, head and neck		+	MTX, LCV	1400	
20	60	71	M	Adenocarcinoma, rectum	+			1400	600 × 5
21	58	66	M	Adenocarcinoma, colon	+			1400	
22	42	56	M	Adenocarcinoma, unknown primary site			VP-16-213, 5FU, BCNU	1400	
23	52	60	M	Epidermoid carcinoma, head and neck		+	MTX	1400	600 × 5
24	49	56	M	Hepatoma	–	–		1400	600 × 5
25	43	75	M	Adenocarcinoma, lung	+		ICRF-159, CYC, CCNU	1400	800 × 5
26	54	55	M	Transitional cell carcinoma, bladder	+	+	IFOS	1400	800 × 5
27	58	62	M	Epidermoid carcinoma, head and neck			MTX	1900	
28	46	49	M	Epidermoid carcinoma, esophagus		+		1900	
29	29	43	F	Adenocarcinoma, colon		+	5FU		1100 × 6
30	57	44	F	Adenocarcinoma, colon	+		5FU		1100 × 6
31	46	59	M	Malignant melanoma			MeCCNU, DTIC		1100 × 6
32	40	42	F	Adenocarcinoma, colon	+	+	5FU, VCR		1100 × 6
33	55	86	M	Squamous cell carcinoma, penis	+	+	BLEO		1100 × 6
34	55	52	M	Epidermoid carcinoma, lung		+	STN		1100 × 6
35	58	56	M	Epidermoid carcinoma, esophagus		+			1100 × 6
36	52	88	M	Adenocarcinoma, colon	+				1100 × 6

*IFOS = isophosphamide; 5FU = 5-fluorouracil; VCR = vincristine; HYDROXY = hydroxyurea; PROCARB = procarbazine; CYC=cyclophosphamide; MTX = methotrexate; LCV = leucovorin; MeCCNU = methyl-CCNU; BLEO = bleomycin; STN = streptozotocin.

TABLE 2.—Toxicity of a single dose of 1070-1900 mg/m² of thalicarpine

Patient No.	Dose (mg/m²)	Toxic effects*						
		Arm pain	CNS depression	Nausea and vomiting	Hypotension	Hypertension	Arrhythmia (PVCs)	EKG changes†
1	1070	+	+	–	–	–	–	–
15		–	–	–	–	–	–	–
16		++	+	–	–	–	–	–
17		+++	+	–	–	–	–	–
12	1400	++	–	–	–	–	–	–
18		++	+	+	–	+	–	+
19		+	++	–	–	–	–	+
20		+	+	–	++	–	–	+
21		+	+	–	–	–	–	–
22		+	++++‡	–	–	–	–	‡
23		–	–	–	+	–	–	+
24		+++	–	–	–	+	–	–
25		+	+	+	–	–	–	–
26		++	+	–	–	–	+	+
27	1900	+++	++	+	–	+		+
28		–	++-+++‡	–	–	–	–	+

*+ = Mild; ++ = moderate; +++ = severe; ++++ = life threatening.
†T-wave depression, widening of QRS, increase in PR interval.
‡See Results section.

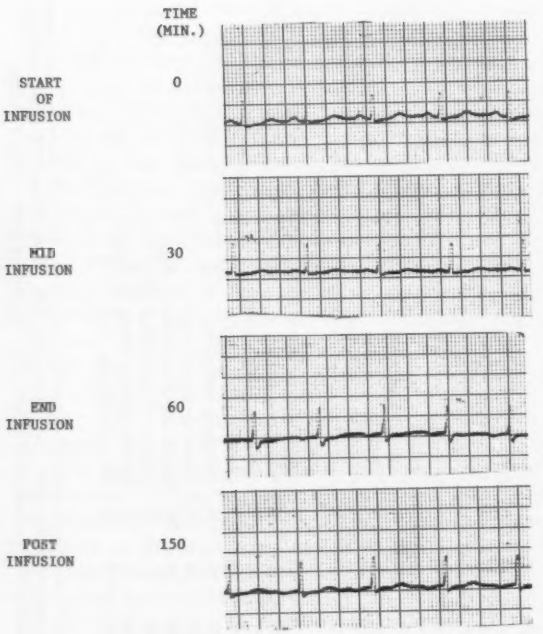

FIGURE 2.—EKG recordings (standard Lead II) taken during and 90 mins after the end of the infusion of 1680 mg (1400 mg/m²) of thalicarpine in 1 liter over 1 hr (patient No. 18).

infusion of 1400 mg/m². EEG recordings for two patients (Nos. 21 and 23) carried out before treatment and 2 hours after infusion of 1400 mg/m² on a Grass Model 6 EEG showed only normal changes of drowsiness and light sleep.

Six patients received five weekly doses of 600 (three patients) or 800 (three patients) mg/m² after receiving 1400 mg/m². Two patients experienced toxic effects which had not occurred after the single dose; patient No. 23 had mild hypotension from 600 mg/m² and patient No. 21 had multiple premature ventricular contractions (PVCs) during the first infusion of 600 mg/m² which necessitated discontinuation of the drug.

Eight patients received 1100 mg/m² weekly × 6. The toxic effects seen in this group are shown in table 3. This dose was well tolerated; no nausea, vomiting, hypertension, or cardiac arrhythmias were seen on this schedule.

There were no drug-related deaths in this study although two patients had reactions which could have been considered life threatening and which might have been related to drug administration. Patient No. 22 was suffering from severe pain for which he was receiving 20 mg of methadone orally every 4-6-hour schedule. He was also occasionally receiving 30 mg of flurazepam orally at bed time. He received a dose of 20 mg of methadone 2

TABLE 3.—Toxicity of a weekly dose of 1100 mg/m² of thalicarpine

Patient No.	No. of weekly doses	Toxic effects*				
		Arm pain	CNS depression	Hypotension	EKG changes†	Flushing, lacrimation, and stuffy nose
29	3	+++	–	–	–	
30	6	++	+	–	+	
31	4	++	+	–	–	
32	6	++	+	–	–	
33	6	–	–	–	–	
34	6	+++	–	–	–	+
35	6	++	–	–	+	
36	6	+	–	++	+	+

*+ = Mild; ++ = moderate; +++ = severe; ++++ = life threatening.
†T-wave depression, widening of QRS, increase in PR interval.

hours before the start of an infusion of 1400 mg/m² of thalicarpine given over a 2-hour period. Five hours after the end of the thalicarpine infusion, the patient was found to be extremely lethargic, not responsive to pain, and barely responsive to verbal commands. Pulse and blood pressure was normal but respirations were shallow, 7 per minute with 15-30-second periods of apnea. The attending physician felt that the patient had marked CNS depression which was most likely related to methadone toxicity enhanced by the cerebral depressant effect of thalicarpine. The patient was developing respiratory arrest and 0.4 mg of haloxone was given iv. The patient responded with diaphoresis, mydriasis, and extreme agitation which were thought to be indications of acute methadone withdrawal, and he was treated with restraint and 2 mg of morphine iv followed by 5 mg of morphine iv. The patient became less restless but a routine followup EKG taken 48 hours after the drug infusion showed early changes of an acute myocardial infarction from which the patient recovered.

Patient No. 28 had hypercalcemia with a serum calcium level of 13.3 mg/100 ml and a serum albumin of 2.8 mg/100 ml. He was also a chronic ethanal abuser and had had numerous prior admissions for this. He received 50 mg of meperidine immediately before the beginning of an infusion of 1900 mg/m² of thalicarpine. The patient slept during the infusion; he was arousable and would answer questions but immediately fell asleep again. After the infusion the patient remained lethargic but was able to respond to verbal commands. He was also hyperactive to stimuli such as pain and touch. This condition persisted in the days after thalicarpine therapy. He continued to have hypercalcemia with the serum Ca^{++} slowly rising over the next 10 days to 16.3 mg/100 ml; his state of mentation continued to be poor. Treatment with intensive iv hydration and steroids was ineffective in lowering the serum Ca^{++} or improving the patient's condition. We thought that his mental state could be due to hypercalcemia, chronic ethanal abuse, metastatic tumor, the effects of thalicarpine, or a combination of these. Because of a deterioration in the patient's condition, he was treated with mithramycin[9] 10 days after treatment with thalicarpine. The serum Ca^{++} fell to 8.1 mg/100 ml with some improvement in his mental status.

DISCUSSION

Thalicarpine is a plant alkaloid that was chosen for phase I clinical study on the basis of its activity against the Walker 256 carcinosarcoma, its novel structure, and the absence of myelosuppressive toxicity in animal testing. Its mode of cytotoxic activity has not been completely elucidated. It binds to DNA (5) and in isolated leukemia L1210 cells it produces an inhibition of DNA, RNA, and protein synthesis, the inhibition of DNA synthesis being the most rapid effect and being only partially reversible (6). The compound has a number of actions on the cardiovascular system. In the anesthetized rhesus monkey it depresses mean arterial pressure but increases carotid artery blood flow and heart rate. It also causes a persistent decrease in coronary perfusion pressure (7).

As noted above, the major preclinical toxic effects were cardiopulmonary and CNS toxicity. However, transient elevations of serum glutamic pyruvic transaminase and SGOT were observed in dogs. The micropathology showed a reduced glycogen content, central congestion, and cloudy swelling of parenchymal cells in the liver and focal cloudy swelling of the proximal tubules in the kidney indicating that the drug had hepatic and renal toxicity (8).

[9]Mithramycin: NSC-24559; CAS reg. No. 18378-89-7.

The only report of preclinical pharmacology indicated that the compound was cleared from the plasma and deposited in tissues within 10 minutes after the injection. About 1% of the drug appeared in the urine. In dogs sacrificed 24 hours after the last dose, the highest concentrations of drug were found in the liver, lung, kidney, pancreas, spleen, and heart (8).

In the first part of the phase I study we investigated the toxicity of a single-dose administration using a starting dose of 200 mg/m^2, approximately one third of the low toxic dose in the rhesus monkey. The rhesus monkey was used rather than the more normal most-sensitive animal species as the basis for calculating the starting dose because we felt that the only meaningful toxicity data were those acquired from 1- or 2-hour infusions and the rhesus monkey is the only animal species for which adequate data on toxicity of infusion are available. The first dose escalation was up to 400 mg/m^2; however, this patient (No. 4) had hypertension and we therefore investigated a dose of 300 mg/m^2 and escalated from this dose. The highest dose reached (1900 mg/m^2) was given to only two patients and the administration of this dose was discontinued because we felt that it was too toxic. We then explored weekly doses and initially gave a loading dose of 1400 mg/m^2, following this by weekly maintenance doses of 600 and then 800 mg/m^2 for five weekly doses. However, when the weekly dose approached the loading dose, the use of the loading dose was discontinued for simplicity.

The only toxic effects seen in this study were acute. No renal, hepatic, or bone marrow toxicity was seen and no long term effects of the drug were observed. (Patients were observed for up to 4 months after receiving thalicarpine.)

The maximum tolerated dose of single-dose administration was 1400 mg/m^2. Except as noted below, this dose appeared to be safe and manageable though considerable drowsiness was seen after it was given in most patients and its administration in the hospital was required. On the weekly dose administration, 1100 mg/m^2 was easily manageable and produced only mild to moderate toxicity. It could be given on an outpatient basis to most patients and is the recommended dose and schedule for phase II testing.

No specific investigations of the mechanisms of the cardiovascular or CNS toxicity of thalicarpine were made during this study. In view of the nature of the EKG changes, measurements were conducted of electrolytes (and specifically potassium) immediately after infusion and at 24 hours after infusion, but these indicated no change. Similarly, measurements of CPK levels were noncontributory. EEG recordings taken in two patients 2 hours after infusion showed only the changes associated with the drowsiness seen with the drug. There were no specific abnormalities. The only significant feature of the CNS toxicity was that two patients who exhibited marked CNS depression both had received a dose of narcotic immediately preceding the administration of thalicarpine. Although these are only two cases and therefore no generalizations are possible (especially since one of the patients had a high serum calcium level undoubtedly contributing to the CNS depression and confusion that were noted in this patient), it might be noted that thalicarpine has been shown to exert a depressant effect on the NADPH and oxygen-dependent microsomal mixed-function oxidase drug-metabolizing enzyme system of the liver (9). Both of the narcotics are extensively metabolized by the liver so that we may be seeing a potentiation of the effect of these narcotics as a result of depression of their metabolism by thalicarpine.

No objective tumor response was seen in our group of patients. However, in view of the complete lack of myelosuppression of this compound, we feel that it warrants phase II testing because of its potential use in combination with myelosuppressive agents if it should be found to have activity in specific tumor types.

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Clinical Phase I Trial of Inosine Dialdehyde (NSC-118994)[1,2,3]

Jerome Kaufman and Arnold Mittelman[4,5]

SUMMARY

An initial clinical phase I trial of inosine dialdehyde has been carried out in 40 patients at dose levels of 30-4000 mg/m² for 5 days given intravenously (iv) monthly. At 1.5 g/m², noncumulative dose-related toxicity occurred in all patients which consisted of nausea and vomiting, local pain, alterations in coagulation mechanism, elevated partial thromboplastin time, and positive Coombs' test. No dose-limiting leukopenia, thrombocytopenia, anemia, or bleeding occurred; however, depression of the leukocyte and platelet counts, and decreased hemoglobin value were observed. The dose-limiting toxic effect was renal tubular damage with reversible acute renal failure in one of four patients who received 3000 mg/m² iv for 5 days. Refractory hypercalcemia was controlled in three of three patients without tumor effect. Responses occurred in patients with seminoma, oat cell carcinoma, and melanoma. A starting dose of 2 g/m² for 3 days monthly is recommended for phase II trials and a trial in lung carcinoma is now being conducted.

[Cancer Chemother Rep 59:1007-1014, 1975]

The obvious importance of purine and pyrimidine bases in all nucleic acid metabolism and nucleotide synthesis has made them attractive foci for a rational attack on neoplastic disease. Many purine and pyrimidine analogs have been synthesized and tested in animal models and in man in a search for effective chemotherapeutic agents. Such useful agents as 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), and 6-thioguanine represent modification of the purine and pyrimidine bases. Modification of the ribose sugar has resulted in the addition of cytosine arabinoside to our therapeutic spectrum.

Inosine dialdehyde (fig 1) represents another approach to the modification of the ribose moiety of nucleosides. Dvonch et al (1) have prepared a variety of purine nucleoside aldehydes by the periodate oxidation of nucleosides. Several of these compounds, among them the oxidation product of β-D-ribosyl-6-methyl-thiopurine, have shown antitumor and immunosuppressive activity (1,2). The periodate oxidation product of inosine, commonly called inosine dialdehyde, was prepared for preclinical evaluation and eventual clinical trial.

Inosine dialdehyde possesses modest antitumor activity in several animal tumor systems: a 122% increase in lifespan (ILS) in AKR leukemia extraperitoneally, a 43% ILS in Lewis lung carcinoma, and activity against L1210 leukemia. It has shown no activity against the Walker 256 sarcoma, the B15 melanoma, or the rodent ependymoblastoma.[6]

The chemical interactions by which inosine dialdehyde exerts its biologic effects are not known. Other purine riboside aldehydes have been studied and some information is available concerning possible mechanisms of action. Since the aldehyde groups are highly reactive, one could expect the formation of Schiff-bases with many types of proteins including enzymes (3,4). Cross-linking of macromolecules by the aldehyde is also possible. There is no evidence to indicate that inosine dialdehyde is phosphorylated although the 5-hydroxy group is intact. One, therefore, would not expect inosine dialdehyde to be incorporated into nucleic acids or to have a specific effect upon the enzymes of de novo purine synthesis or upon the salvage pathway, despite the compounds derivation from inosine. It is probably not a substrate for xanthine oxidase because of the aldehyde

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[2]Supported in part by grant RR-262 from the General Clinical Research Centers Program, Division of Research Resources, National Institutes of Health (NIH); and by Public Health Service grant CA-12296 from the National Cancer Institute (NCI), NIH, Department of Health, Education, and Welfare.

[3]Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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[6]Cysyk RL, and Adamson RH. In preparation.

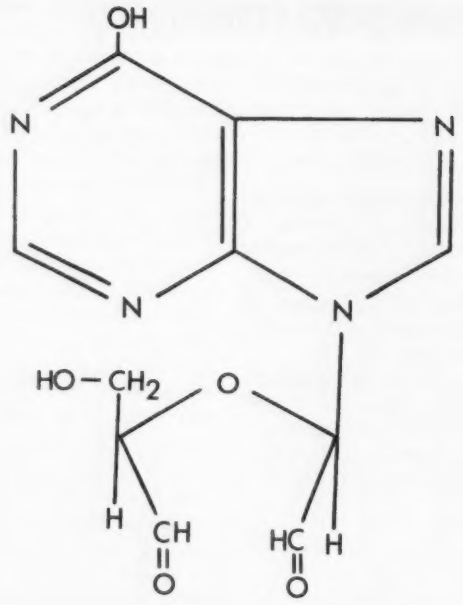

FIGURE 1.—Chemical structure of inosine dialdehyde.

grouping. The work of Cysyk (5) indicates that the compound may be excreted in urine, quantitatively unchanged. The data of these investigators as well as our own data indicate that inosine dialdehyde does bind to plasma proteins.

STUDY PROGRAM

Preparation of Inosine Dialdehyde and Mode of Administration

Inosine dialdehyde was supplied in 500-mg flint vials. The dry compound was reconstituted with 9.5 ml of sterile H_2O, was diluted with 1000 ml of 5% dextrose in water, and was given intravenously (iv) over a 6-hour period.

Patients

Forty patients were treated with varying dose schedules of inosine dialdehyde given iv. All of the patients were hospitalized; their cases were initially evaluated and subsequently followed at Roswell Park Memorial Institute. All but one of the patients had received every available therapy for their metastatic disease before entering this study, and all of the patients had had a treatment-free period of at least 1 month. Patients were not excluded because of hematologic depression or impaired renal function.

Dose Schedule

The toxicologic data supplied by the Division of Cancer Treatment, NCI, revealed a consistent LD50 between dogs and monkeys. The dog was most sensitive with an LD50 of 400 mg/m² for 5 days, given iv. At doses above 400 mg/m² for 5 days, the monkey exhibited liver necrosis and renal injury. An initial dose of 30 mg/m² given daily for 5 days every 10 days seemed a safe program. It was planned to enter two patients into each dose category. The following schema was followed: 30-60 mg/m² (one patient); 90-120 mg/m² (two patients); 240 mg/m² (two patients); and 480 mg/m² (two patients). No toxicity was observed in any of these patients.

The protocol was then modified: four patients received 1 g/m², seven received 1.5 g/m², eight received 2.0 g/m², and four received 3.0 g/m². All patients received five daily doses iv every 10 days for three courses or until all signs of toxicity remitted. In no patient was stepwise escalation of the dose carried out.

Study Parameters

A bone marrow biopsy was performed on each patient entered into the study. The following determinations were obtained at least three times a week from each patient during the treatment.

Hematologic.—A complete blood count including hemoglobin, white blood cell count (wbc) with differential, platelet count, prothrombin time, and augmented partial thromboplastin time (APTT).

Hepatic function.—Bilirubin, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase, albumin, and globulin values.

Electrolytes.—Sodium, potassium (carbon dioxide), calcium, and phosphorus levels.

Renal function.—Blood urea nitrogen (BUN), creatinine, uric acid, urinalysis, creatinine clearance, total urinary sodium, potassium, protein, calcium, and phosphorus values.

RESULTS

Determination of the Biologically Active Dose

Patients who received 30, 60, 90, or 120 mg/m² for 5 days iv every 10 days for at least three courses exhibited no biologic effect from inosine dialdehyde. Patients receiving 240 or 480 mg/m² for 5 days iv exhibited mild to moderate nausea and occasional vomiting beginning 1-2 hours after the start of medication. In two patients nausea continued for up to 12 hours after the end of the infusion. There was mild to moderate muscle and bone pain in the arm and

shoulder of the infused arm. There was no observable effect on any of the hematologic or serum chemistry parameters studied. Four patients received a dose of 1 g/m² for 5 days every 10 days. No systemic toxicity other than nausea and vomiting was observed.

Seven patients received 1.5 g/m² for 5 days iv every 10 days. Once again there was nausea and vomiting, phlebitis, and muscle pain as well as the only occurrence of a 1-2-mm papular blanching pruritic rash on the infused arm during the infusion which spontaneously faded 2 hours after medication. This rash did not recur with subsequent doses. There was no observed hematologic or renal toxicity. All patients in this group have either had progression or no change in their disease status though subjectively we saw a decrease in pain and an improvement in performance status in several patients (table 1).

Eight patients (table 2) received 2 g/m² iv for 5 days every 10 days or until all signs of toxicity remitted. In practice, this became a monthly course for all eight patients. All patients had prior standard treatment except for a 43-year-old man with seminoma.[7] All patients had moderate to severe nausea and vomiting, arm pain, and phlebitis. For the first time, local and systemic blisters resembling a second-degree burn were seen on the infusion arm as well as on the skin overlying pressure points in the patient's body. These blisters were best treated with cold compresses and exacerbated with heat. Buffering of the administered solution to pH 7.4 eliminated this problem. Reversible renal and hematologic toxic effects were also observed (figs 2 and 3).

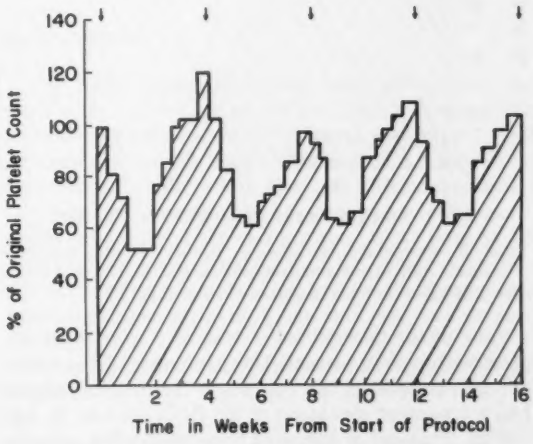

FIGURE 2.—Percentage variation in median platelet counts of eight patients who received inosine dialdehyde at a dose level of 2 g/m² for 5 days repeated every 28 days (↓).

[7]Initial biopsy indicated undifferentiated carcinoma. After a complete clinical remission a diagnosis of seminoma was made on a retroperitoneal biopsy.

TABLE 1.—Clinical characteristics of patients who received 1.5 g/m² of inosine dialdehyde

Diagnosis	Treatment time (days)	No. of courses	Total dose (g)	No. of patients experiencing toxic effects						Response¶
				Skin	Pain	Nausea and vomiting	Diarrhea	Renal	Hematologic	
Leiomyosarcoma	20	2	22.5	0	0	3	3	1*	0	NC
Large cell carcinoma	70	4	46	0	3†‡	2	0	0	0	P
Cancer of the colon	30	3	35	0	2†‡	3	3	0	0	NC
Synovial sarcoma	30	3	40	2§	2†‡	2	0	0	0	P
Leiomyosarcoma	20	2	22.5	0	0	1	0	0	0	NC
Acute lymphoblastic leukemia	18	2	9	0	0	3	0	0	0	NC
Renal cell carcinoma	36	2	24	0	2†	2	0	0	0	NC

*Transient proteinuria after each course.
†Phlebitis with abscess at iv infiltration site.
‡Muscle and bone pain in the infused arm.
§Rash, pruritis.
¶Response: NC = no change; P = progression.

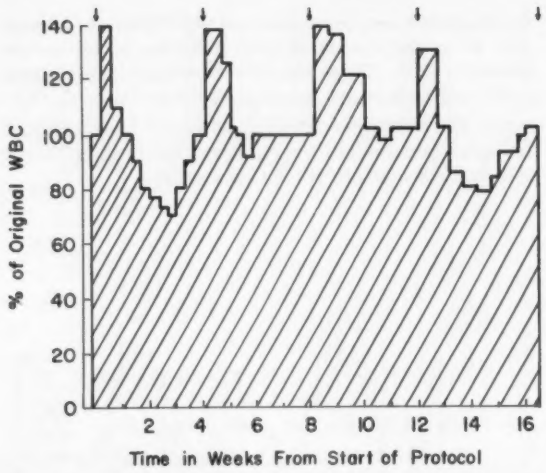

FIGURE 3.—Percentage variation in median of total leukocyte counts in eight patients who received inosine dialdehyde at a dose level of 2 g/m² for 5 days repeated every 28 days (↓).

Four patients received a dose of 3 g/m². One patient with widely metastatic carcinoma of the stomach who completed two courses of inosine dialdehyde had a transient elevation of his BUN level to 23 mg/100 ml. This value returned to normal after completion of therapy. One patient with carcinoma of the breast had a grand mal seizure, became comatose, and died while receiving her initial course of inosine dialdehyde. A postmortem examination revealed the presence of extensive brain metastases. The third patient in this group was in an accelerated phase of uncontrolled "blastic crisis" of chronic myelogenous leukemia (95% blast cells in peripheral blood) and died of clostridial sepsis after three doses of inosine dialdehyde. The fourth patient who received 3 g/m² completed his first course of therapy without signs of acute renal or hematologic toxicity but died 2 days later of massive hematemesis from his carcinoma of the stomach.

We are not able to evaluate the effect of the dose of 3 g/m² for 5 days in the latter three patients; however, acute reversible renal insufficiency developed in the first patient discussed. We must assume that such toxicity is likely in any patient receiving 3 g/m² for 5 days. One patient with widespread metastatic renal cell carcinoma received 4 g/m² for 5 days. At the end of treatment the patient was azotemic (BUN = 120 mg/100 ml) and died 6 days after receiving his last dose. A postmortem examination revealed severe, diffuse renal tubular necrosis. This was the only unequivocal drug-related death in our study.

Systemic Toxicity at 2 g/m² for 5 Days

Renal

The major dose-limiting toxic effect was renal tubular damage. At a dose level of 1.5 g/m² daily for 5 days, occasional transient proteinuria was observed

TABLE 2.—Clinical characteristics of patients who received 2 g/m² of inosine dialdehyde

Patient No.	Diagnosis	Treatment time (days)	No. of courses	Total dose (g)	Toxic effects—type and degree of severity				Response*
					Skin	Pain	Nausea	Vomiting	
1	Seminoma	110	5	101	None	Slight	Moderate	Mild	CR
2	Cancer of the breast	26	2	13.5	Moderate	Moderate	Mild	Mild	Control of hypercalcemia
3	Sarcoma (undifferentiated)	40	3	24	None	Slight	Moderate	Moderate	NC
4	Malignant melanoma	10	1	14	None	Slight	Slight	Slight	NC
5	Cancer of the colon	27	2	24	None	Slight	Moderate	Moderate	NC
6	Cancer of the breast	35	2	30	None	Moderate	Moderate	Moderate	NC
7	Synovial sarcoma	31	2	18	Moderate	Moderate	Slight	Slight	P
8	Squamous cell carcinoma	26	2	20	Mild	Slight	Slight	Slight	Control of hypercalcemia

*Response: CR = complete response; NC = no change; P = progression.

without any change in creatinine clearance. At 2 g/m^2 daily for 5 days, patients had significant proteinuria by the 4th day of treatment which had returned to normal 2 weeks after the drug course had ended. This toxicity was not cumulative. One patient had a trace of protein in a 24-hour urine specimen at the start of the seventh treatment course, 200 days after the first dose of inosine dialdehyde. In this same patient-group there was no significant decrease in creatinine clearance even in patients with pretreatment levels less than 40 ml/minute. It generally took 3-4 weeks for all of the manifestations of renal toxicity to remit.

Hematologic

Platelet count.—A rapid depletion of platelets could be observed at the end of a treatment course of 2 g/m^2 for 5 days with return to pretreatment levels by 28 days (fig 2). In only one patient was the platelet count nadir less than 100,000 platelets/mm^3.

Wbc.—Initial elevation of the leukocyte count, 2-3 days after the start of treatment, was followed by a mild leukopenia (3000-4000 cells/mm^3) in one half of the patients receiving 2 g/m^2. The leukopenia was largely a neutropenia. Complete recovery was seen 3 weeks after completion of the 5-day course (fig 3).

Hemoglobin.—A Coombs' positive hemolytic anemia with a depression of 1-2 g/100 ml in the hemoglobin level after the first course was almost a constant finding (6). This anemia was not cumulative with repeated courses, though the hemoglobin concentration did not return to pretreatment levels while the patients were receiving treatment (fig 4).

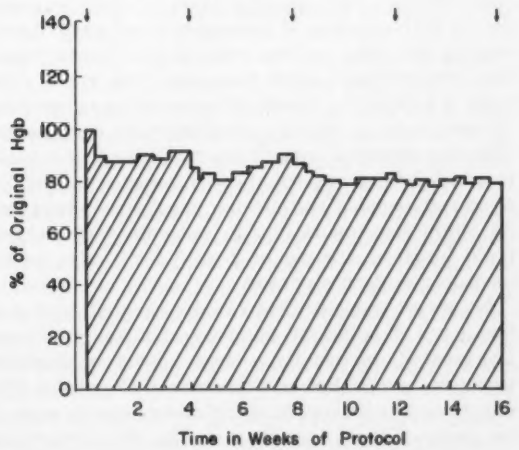

FIGURE 4.—Percentage variation in median hemoglobin levels of eight patients who received inosine dialdehyde at a dose level of 2 g/m^2 for 5 days repeated every 28 days (↓).

Coagulation.—At 3-5 days after the start of inosine dialdehyde administration, at a dose level of 1.5-2.0 g/m^2 for 5 days, APTT became elevated with a return to normal levels by 5 days after the end of drug infusion. Further investigation has indicated that this is a drug-related defect in fibrinogen polymerization occurring within 6 hours after the start of inosine dialdehyde.[8] No significant bleeding has been observed.

Gastrointestinal

The major gastrointestinal toxic effect was the nausea and vomiting which occurred 2 hours after the start of infusion in all patients receiving 1 g/m^2 or more in five daily doses. This reaction could not be completely controlled by the use of chlorpromazine or prochlorpromazine. An occasional patient experienced severe retching and nausea for as long as 12 hours after each treatment. This effect was not dose-related above the 1-g/m^2 level. Stomatitis, pharyngitis, or hepatic injury was not seen at any dose level.

These systemic toxicities did not appear to be cumulative.

Regional Toxicity at 2 g/m^2 for 5 Days

All patients receiving an unbuffered dose of 2 g/m^2 had evidence of severe, localized venospasm and venous thrombosis at the site of infusion. Extravasation of inosine dialdehyde at this concentration caused erythemia, warmth, pain, and subsequent subcutaneous necrosis and sloughing. These problems were overcome by buffering the inosine dialdehyde solution to a pH of 7.4. Such buffered solutions were safely and easily administered through a peripheral vein via a small bore needle. Extravasation of such a neutral solution caused no tissue damage.

Effect of Inosine Dialdehyde on Calcium Metabolism

Three patients who were hypercalcemic prior to receiving inosine dialdehyde therapy became normocalcemic after receiving 2 g/m^2 of the drug daily for 3-5 days. The hypercalcemia of these patients could not be controlled by adequate hydration and adrenocortical steroids. One patient had carcinoma of the breast with extensive osseous metastases; the other had squamous cell carcinoma of the lung and no osseous metastases. The third patient had hypercalcemia due to extensive osseous metastases from a renal cell carcinoma. Reduction in urinary calcium

[8]The mechanism of this alteration is now being investigated.

accompanied the return of serum calcium to normal levels and was seen promptly after the 2nd day of therapy. The transient increase in urinary calcium seen after the first dose was also observed in normocalcemic patients. A return of serum calcium values to normal levels occurred somewhat more slowly. Hypercalcemia recurred prior to the second course of inosine dialdehyde in the third patient discussed above. Once again, therapy resulted in a return of serum calcium to normal levels and in a decrease in urinary calcium.

Biologic Effects of Inosine Dialdehyde at a Safe, Therapeutic Dose—2 g/m² for 3 Days Given iv Every 4 Weeks

Ten patients were treated on this program (table 3) for a period of from 24 days to 4 months. Using the buffered solution, no regional or dermatologic toxic effects were seen. Transient reversible urinary proteinuria was observed but no changes in BUN levels or creatinine clearance were seen. Hematologic toxicity was confined to mild leukopenia (2500-3000 cells/mm³) and thrombocytopenia (100,000-150,000 platelets/mm³) with the nadir seen 10 days after cessation of therapy. All hematologic and renal functions returned to normal before the subsequent monthly dose was given.

Partial responses were seen in two patients: one with oat cell carcinoma of the lung and one with malignant melanoma. The patient with oat cell carcinoma had a partial regression of multiple skin and pulmonary lesions beginning 10 days after the start of treatment and lasting for 50 days. The patient with melanoma had complete disappearance of previously intractable ascites and relief of obstructive jaundice for 90 days.

In three of eight normocalcemic patients who received inosine dialdehyde (2 g/m² daily for 5 days monthly) a fall in the serum calcium levels from 0.5 to 1.0 mg/100 ml was observed. Tetany was not seen in any patient though it has been described in children.[9] The objective response of the tumor could not be correlated with the control of hypercalcemia, although control of hypercalcemia was associated with the stability of the metastatic disease.

TABLE 3.—Biologic effects of inosine dialdehyde at a dose level of 2 g/m² for 3 days given iv monthly

Disease	Time on protocol (days)	Patient response
Leiomyosarcoma	24	Died of disease
Chondrosarcoma	10	No change
Melanoma	107	Partial response; control of hypercalcemia
Renal carcinoma	90	No change
Colon carcinoma	60	No change
Colon carcinoma	64	No change
Colon carcinoma	67	No change
Oat cell carcinoma, lung	70	Partial response
Leiomyosarcoma	30	Progression
Leiomyosarcoma	42	Died of disease

DISCUSSION

The primary purpose of this study was to determine the maximum tolerated dose of inosine dialdehyde and to delineate the human toxicology. Clinical responses in various tumor types were noted (table 4) but no attempt at comparing response rates to conventional drugs was made. Inferences drawn from these results should be made with caution.

The fact that a complete response in one patient with seminoma and partial responses in one patient with oat cell carcinoma and in one patient with melanoma were observed indicates that further study of the effect of inosine dialdehyde in the treatment of these tumors is warranted.[10]

There was no relationship between the response to treatment and the degree of hematologic or renal toxicity. At doses of 2 g/m² for 3 days, the hematologic toxicity was predictable and was not dose-limiting. The positive Coombs' test was observed within 3 days of treatment in all patients receiving the drug and the test did not become negative until 60 days after treatment was ended. The 3-day schedule was chosen because of the steep slope of renal tubular toxicity manifested by proteinuria that was observed in patients receiving 2 or 3 g/m². It was felt that in patients with severe prerenal or renal compromise, the effect of high serum-drug levels might be cumulative. In patients with normal renal function, the use of 2 g/m² for 5 days would seem only moderately toxic.

No severe toxicity that required transfusion was noted but if sufficient numbers of anemic patients are treated, eventually a transfusion requirement might be established in a fraction of the group. The effects on the leukocyte and platelet counts were of no major clinical importance. The effect on blood

[9]Karon M. Personal communication.

[10]A phase II study in lung carcinoma is now in progress.

TABLE 4.—Clinical responses in various tumor types to inosine dialdehyde (2 g/m² for 3-5 days monthly)

Tumor type or site	No. of responders/ total No. of patients evaluable	Comments*
Breast	0/4	Control of hypercalcemia, 1 of 4 patients
Colon	0/3	
Malignant melanoma	1/1	PR after 2 g/m² for 3 days; liver (span, 20 cm) stable for 4 mos
Large cell carcinoma	0/1	
Oat cell carcinoma	1/1	PR after 2 g/m² for 3 days
Squamous cell carcinoma	0/1	Control of hypercalcemia
Renal cell carcinoma	0/2	Control of hypercalcemia, 1 of 2 patients
Seminoma	1/1	CR (clinical) after 2 g/m² for 5 days
Chondrosarcoma	0/1	
Leiomyosarcoma	0/1	
Liposarcoma	0/2	1 NC for 7 mos after 2 g/m² for 5 days
Synovial sarcoma	0/1	
Total	3/19	1 CR + 2 PRs

*Response: PR = partial response; CR = complete response; NC = no change.

clotting occurred within 6 hours of the start of drug administration and manifested itself as an elevation of the thrombin and the reptilase times.[11] These effects are not reversible with protamine or normal plasma. Since the serum fibrinogen level was elevated in these patients, our working assumption is that this was an effect on fibrinogen polymerization. This would seem to differ from the effects of mithramycin which acts on depressing factors II and VII (7).

Lowering of the serum calcium level occurred within 5 days from the start of inosine dialdehyde administration and did not appear to represent excretion in the urine; the possibility of stool excretion of calcium was not investigated but this is usually not a pathway of calcium loss except in patients with biliary fistula. Since there was no relationship between tumor response and the depression of serum calcium levels, an independent effect on bone metabolism such as has been observed with mithramycin (8) may be a possibility.

Some possible clinical usefulness for inosine dialdehyde is indicated by the objective response to therapy seen in several patients with neoplasms. Since the major toxicity is renal rather than hematologic, its potential use in combination chemotherapy may bear investigation. For these reasons we feel this agent deserves a phase II clinical trial.

The mechanism of action of inosine dialdehyde is not clearly understood. Its ability to cross-link proteins may explain its effect on the coagulation mechanism. Its effect on calcium metabolism and renal function needs further investigation. We do know that nontoxic doses will enhance renal plasma flow in dogs.[12] Toxic levels, ie, 60 mg/kg daily for 3 days, will diminish renal plasma flow by over 50%. This may account for the renal toxicity seen in man as well as in dogs. The mechanism of antitumor activity can only be guessed at. The lack of significant leukopenia, thrombocytopenia, or intestinal mucosal

[11]Kaufman J, Mink IB, and Mittelman A. In preparation.

[12]Keogh JB, Kaufman J, and Mittelman A. Effect of diglycoaldehyde on renal blood flow of dogs. In preparation.

injury is against alkylation as a likely mechanism of antitumor activity.

ADDENDUM

Any patient with a history of radiation treatment to the kidney, diabetic-nephropathy, or other renal disease should receive an initial dose of 1.5 g/m^2 × 3 days instead of 2 g/m^2 × 3 days if creatinine clearance is below 60 cc/minute.

Subsequently we have observed reversible renal failure 2 days after treatment in two patients. Both patients had creatinine clearance below 40 cc/minute. One patient with active pyelonephritis had received total abdominal radiation treatment with 2500 rads of renal radiation. The other patient had diabetic glomerulonephritis and renal melanoma.

LIST OF COMPOUNDS

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU

Inosine dialdehyde: NSC-118994; diglycolaldehyde, α-(hydroxymethyl)-α′-(6-hydroxy-9*H*-purin-9-yl)-; diglycoaldehyde

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

Mithramycin: NSC-24559; CAS reg. No. 18378-89-7

6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-

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Use of Combination Adriamycin (NSC-123127) and DTIC (NSC-45388) in Children With Advanced Stage IV Neuroblastoma[1,2]

Sanford Leikin, Irwin Bernstein, Audrey Evans, Jerry Finklestein, Robert Hittle, and Martin Klemperer[3,4]

SUMMARY

Adriamycin and DTIC were used in combination because of their reported effectiveness in neuroblastoma when administered as single agents and because of the poor survival rate of patients with this malignancy in its disseminated stage. Eighteen patients with previously treated stage IV neuroblastoma received this combination chemotherapy every 21 days. Two to eight courses were administered. Two partial and no complete remissions were seen. Mild to moderate gastrointestinal and hematologic toxicity was observed.

[Cancer Chemother Rep 59:1015-1018, 1975]

Children with stage IV neuroblastoma are defined as having remote disease which involves the skeleton, soft tissues, and distant lymph nodes (1). More than 50% of patients with neuroblastoma are in this category at the time of diagnosis. Despite the availability of a number of chemotherapeutic agents, including cyclophosphamide and vincristine, which cause tumor regression in this disease, the overall survival rate of children with metastatic neuroblastoma has not changed appreciably in the last 15-20 years (2). Breslow and McCann found that 2.5% of 118 children with stage IV disease who were more than 1 year old survived, tumor free, for 2 years after diagnosis (3).

Adriamycin and DTIC are agents that have recently been under clinical investigation. Both drugs, used as single agents, have caused regression in several different types of tumors (4-14), including neuroblastoma. Sixteen of 32 patients who had not previously received daunorubicin responded to adriamycin (8-12). Ragab et al (13) have reported responses in nine of 18 patients with neuroblastoma; however, it is not known whether or not these patients had received prior therapy. Three of 13 children with advanced metastatic neuroblastoma demonstrated partial or complete disappearance of lesions after receiving DTIC (14).

Since single agents have not been very effective in stage IV neuroblastoma, it was thought that combination chemotherapy might possibly be more effective. We therefore planned to employ the combination of adriamycin and DTIC in a phase II trial in patients with stage IV neuroblastoma who had become unresponsive to presently accepted standard therapy (cyclophosphamide with or without vincristine).

Previously, the Southwest Oncology Group had employed a dosage schedule of the adriamycin and DTIC combination which was well tolerated and

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[2]Adriamycin: CAS reg. No. 23214-92-8.

DTIC: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide.

[3]This study was done by the Children's Cancer Study Group. The following institutions (with investigators and grant support) participated in this study: Children's Hospital of Washington, DC (*S. Leikin* and *N. Movassaghi*, CA-03888); Children's Hospital of Los Angeles, Calif (*G. Higgins, C. Hyman, J. Ortega, N. Shore, K. Williams, S. Siegel*, and *M. Karon [deceased]*, CA-02649); University of Southern California, Los Angeles (*D. Hammond* and *J. Weiner*, CA-13539); Babies Hospital, New York, NY (*J. Wolff* and *A. Sitarz*, CA-03526); Children's Hospital of Pittsburgh, Pa (*V. Albo, W. Prin*, and *S. Orlando*, CA-07439); Children's Hospital of Columbus, Ohio (*W. Newton* and *I. Ertel*, CA-03750); Children's Orthopedic Hospital, Seattle, Wash (*R. Chard, J. Hartmann*, and *I. Bernstein*, CA-10382); University of Wisconsin, Madison (*P. Joo*, CA-05436); University of Minnesota, Minneapolis (*M. Nesbit* and *W. Krivit*, CA-07306); Children's Memorial Hospital, Chicago, Ill (*W. Borges, A. Schwartz, M. Pierce, J. Nicklas*, and *E. Hackl*, CA-07431); University of Utah Medical Center, Salt Lake City (*E. Lahey* and *J. Wilson*, CA-10198); University of Rochester, NY (*M. Klemperer*, CA-11174); Milwaukee Children's Hospital, Wis (*L. Thatcher* and *S. McCreadie*, CA-11075); Children's Hospital of Philadelphia, Pa (*M. Donaldson, A. Evans*, and *H. Carim*, CA-11796); Cornell Medical Center, New York, NY (*D. Miller*, CA-14557); Indiana University, Indianapolis (*R. Baehner* and *R. Weetman*, CA-13809); New Jersey College of Medicine, Newark (*L. Vitale*, CA-12637); Harbor General Hospital, Torrance, Calif (*J. Finklestein, L. Sieger, L. Greenberg*, and *V. Medrano*, CA-14560); University of British Columbia, Vancouver (*M. Teasdale*, Vancouver Foundation); Princess Margaret Hospital, Toronto, Canada (*M. Sonley*, Ontario Cancer Treatment and Research Foundation grant 41-17); and University of Michigan, Ann Arbor (*R. Heyn*, CA-02971). All CA grants are from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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effective in a group of patients with sarcoma (7). Their treatment regimen was adopted for our study.

MATERIALS AND METHODS

Our study was open to patients under 16 years of age who had stage IV neuroblastoma and were expected to survive a minimum of 1 month. Diagnostic criteria for entry into the study included histologic verification of tumor type and/or demonstration of clumps of tumor cells in the bone marrow with elevated urine catecholamines or their breakdown products, vanillmandelic acid (VMA) and homovanillic acid (HVA). The patients must have demonstrated resistance to chemotherapy but must not have received either DTIC, adriamycin, or daunorubicin. Informed consent was required from the parents or legal guardians prior to entry into this study.

Only patients with a measurable lesion(s) were accepted into this study. Bone marrow involvement was considered a measurable lesion. Patients were required to have an absolute neutrophil count greater than 1500 cells/mm^3 and a platelet count greater than 75,000 platelets/mm^3 unless tumor infiltration of the marrow was present (in which case any neutrophil or platelet count was acceptable).

The following determinations were performed at the time of entry in the study: physical examination, complete blood cell count including platelet count or estimation, bone marrow aspiration, chest roentgenograms plus appropriate additional roentgenographic studies to document the extent of disease, serum glutamic oxaloacetic transaminase (SGOT) and blood urea nitrogen (BUN) values, electrocardiogram (EKG), and quantitative estimations of VMA, HVA, or other catecholamine determinations. Directly measurable lesions were recorded on forms and flow sheets prepared by the Children's Cancer Study Group (CCSG).

Followup examinations to evaluate toxicity and response were required weekly after the first course of therapy and then every 3 weeks thereafter. At each of these visits a complete blood count including a platelet count was performed. BUN and liver function tests were required at 6 weeks, 12 weeks, and every 12 weeks thereafter. If the bone marrow was initially involved, repeat aspirations were also required at these same intervals. Radiologic examinations of involved areas were performed as required. Urinary catecholamines and EKGs were performed every 12 weeks.

The initial drug doses were 60 mg/m^2 for adriamycin given intravenously (iv) on Day 1 and 250 mg/m^2/day for DTIC given iv for five doses. Treatment courses were repeated every 21 days whenever possible. Dose modifications were based on the absolute neutrophil and platelet counts, the state of the bone marrow just prior to the course of treatment, and the degree of hematologic toxicity noted after the previous course of the combination therapy.

A minimum of two courses, or one course in the face of progressive disease, was required for a patient to be considered evaluable. This study was designed to analyze only complete and partial remissions. A complete remission was defined as the disappearance of all evidence of disease for 28 days or more; a partial remission was defined as a greater than 50% decrease in the size of all measurable lesions for 28 days or longer. Disappearance of all tumor cells from the bone marrow was required to establish a response for that index lesion.

RESULTS

Eighteen patients with stage IV neuroblastoma were entered into this study between September 1973 and July 1974. All were considered evaluable. Table 1 gives the patients' age, sex, prior chemotherapy and radiotherapy at the time of entrance into the study, number of courses of adriamycin and DTIC given, maximal drug doses administered, response, and survival duration subsequent to entry in this study.

All of the patients had previously received cyclophosphamide and all but two had received vincristine. In addition, actinomycin D had been administered to one patient, methyl-CCNU to one, and bacillus Calmette-Guérin to another. Sixteen patients had received radiotherapy to their primary or metastatic lesions for palliative purposes. Clumps of tumor cells were found in the bone marrow of eight patients.

There were no complete remissions. Two patients experienced partial remissions. In one child the remission consisted of a reduction in the size of lymph nodes, tumor masses, proptosis, and bone lesions of 2 months' duration. In the other child there was a decrease in the size of bone lesions for 2 months. A third patient demonstrated a disappearance of tumor cells from the bone marrow although a complete or partial remission was not observed since lytic bone lesions persisted. The median duration of survival after entry into the protocol was 3 months with a range of 1.5-8 months.

The toxicity was limited to the gastrointestinal tract and the hematopoietic system. All of the patients experienced nausea and vomiting. This was particularly marked on the day of the injections of both adriamycin and DTIC. DTIC infusions given on the following 4 days alone were generally better tolerated. In every patient there was a prompt decrease

TABLE 1.—Pretreatment data and results of therapy in 18 evaluable patients

Patient No.	Age (yrs)	Sex	Prior chemotherapy with cyclophosphamide and vincristine	Prior radiation therapy	No. of courses of chemotherapy given	Maximal dose given (mg/m²)		Response	Survival duration (mos) from start of study
						Adriamycin	DTIC		
1	3 3/12	F	Yes	No	5	90	350	NR	6
2	1 1/12	M	Yes	Yes	2	60	250	NR	5
3	5 3/12	M	Yes (+ BCG)	Yes	2	60	250	NR	2
4	1 2/12	F	Yes	Yes	2	60	250	NR	3.5
5	4	F	Yes	Yes	4	60	250	NR	2.5
6	3 4/12	M	Yes	Yes	3	40	250	NR	2.5
7	4 10/12	M	Yes (+ methyl-CCNU	Yes	2	60	250	NR	2
8	2 4/12	M	Yes	Yes	3	75	300	PR	2
9	8 4/12	F	Yes	Yes	5	75	300	PR	4
10	2 9/12	M	Yes	Yes	3	60	250	NR	3
11	4 11/12	M	Yes (+ cytosine arabinoside)	Yes	8	75	250	NR	4
12	2 4/12	F	Yes	No	2	60	250	NR	1.75
13	1 8/12	M	Yes (+ methotrexate)	Yes	3	60	250	NR	3
14	3 5/12	M	Yes	Yes	1	60	250	NR	1.5
15	2 5/12	M	Yes (+ actinomycin D)	No	5	60	250	NR	8
16	3 1/12	M	Yes	Yes	6	60	250	NR	5
17	3 2/12	M	*	Yes	4	90	300	NR	2.25
18	3 6/12	M	*	Yes	5	75	300	NR	5

*Cyclophosphamide only.

in the granulocyte and platelet counts after the initiation of each course with a return to pretreatment levels within 21 days. Thirty percent of the courses were associated with either grade 3+ or 4+[5] hematologic toxic effects at their nadirs. There was no evidence of cumulative hematologic toxicity.

Because of the reported adriamycin cardiac toxicity associated with total cumulative doses in excess of 550 mg/m², we planned to discontinue the drug in patients when that dose was reached. However, the maximum total dose administered to any one patient was 480 mg/m² and the average dose was 193 mg/m². Clinical or EKG evidence of cardiac toxicity was not observed in any of the patients. One patient died suddenly (No. 9) 9 days after the completion of her fifth course of adriamycin and DTIC. *Pneumocystis carinii* infection of the lungs was found on autopsy.

DISCUSSION

Survival can be prolonged in patients with stage IV neuroblastoma who respond to chemotherapy (2). Therefore, one of the objectives of the CCSG is to increase the remission rate of newly diagnosed patients with stage IV neuroblastoma from its present level of approximately 40% (2) by identifying new chemotherapeutic agents or combinations active against this neoplasm. This study evaluated a new drug combination in patients with advanced disease. It is disappointing that the two drugs employed in this study which have activity in children with this tumor when used as single agents did not elicit an improved response rate when combined. It is possible that the patients' prior chemotherapy may have facilitated the growth of resistant clones of tumor cells. This is supported by the observation in another study by the CCSG (15) that the addition of DTIC increased the complete and partial remission rate from 40% to 70%. Moreover, the poor general physical condition of these patients as indicated by their short survival duration may have obscured some small additive or synergistic effects. On the other hand, it is possible that these drugs are antagonistic to each other, though this appears unlikely since this has not been previously noted when these drugs were used in other combinations. In fact, DTIC and adriamycin are additive when they are used in combination for sarcomas (11). It should be mentioned that Cangir et al (16) have reported seven complete plus partial responses in 16 patients treated with a combination of adriamycin and DTIC. Since it is not known what prior treatment these patients had received, if any, it is difficult to compare our results with theirs.

[5]3+ Hematologic toxicity = absolute neutrophil count of 250-499 cells/mm³; 4+ hematologic toxicity = absolute neutrophil count <250 cells/mm³.

Although hematopoietic toxicity was not a limiting factor in this study it is doubtful whether increased dosages of these drugs could have been administered since the granulocyte and platelet nadirs were low and ten patients had absolute granulocyte counts of less than 1000 cells/mm^3 after one or more of their courses. One patient died of overwhelming infection at the time of the granulocyte nadir. The frequent occurrence of bone marrow tumor infiltration in this group of patients probably contributed to the hematologic suppression. This is supported by the fact that there is less incidence (and degree) of granulocytopenia and thrombocytopenia in sarcoma patients without bone marrow involvement who are given similar therapy (7). Gastrointestinal toxicity, although frequent, was not a problem. No cardiac toxicity was noted, but only one patient received a total dose that approached the toxic range.

The occurrence of a case of pulmonary pneumocystis infection was not surprising, particularly in light of the immunosuppressive treatment. However, recent observation of *P. carinii* infection in a number of neuroblastoma patients receiving DTIC suggests the possibility of a specific relationship with that drug (15). Further observation is needed to determine the exact relation of this drug and that particular infection.

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Evaluation of Sialogogic Action of Cyclocytidine (NSC-145668) and Anhydro-ara-5-fluorocytidine (NSC-166641)[1,2,3]

Brief Reports and Preliminary Communications

Charlotte A. Schneyer[4] and Wayne M. Galbraith[5,6]

Cyclocytidine produces undesirable side effects that do not preclude its use but do limit its usefulness. These toxic effects include sialorrhea, parotid pain, and postural hypotension (1,2). We recently reported findings which suggest that cyclocytidine has an action on the β-adrenergic receptors and that the undesirable side effects of the drug probably stem chiefly from its β-adrenergic properties (3).

Anhydro-ara-5-fluorocytidine, an agent that differs structurally from cyclocytidine only because it possesses a 5-fluoro atom in the pyrimidine moiety, is currently being evaluated (4) as an antitumor agent. Since these two agents are structurally similar, it is necessary to determine whether anhydro-ara-5-fluorocytidine also has toxic effects similar to those exhibited by cyclocytidine.

Preliminary studies revealed that the intraperitoneal administration of 400-600 mg/kg of anhydro-ara-5-fluorocytidine did not induce salivary secretion in adult rats, at least for the first 90 minutes after injection. Thereafter, only occasional drops of saliva were observed at the end of the salivary ducts and the flow of saliva was infinitesimal or absent. Thus, the agent is virtually inactive as a sialogogue. Equal molar doses of cyclocytidine caused significant secretion from the submaxillary and parotid glands of rats. Anhydro-ara-5-fluorocytidine also appears to be devoid of β-adrenergic properties, at least when gland amylase is used to measure this property. Two hours after stimulation with 400, 500, or 600 mg/kg of anhydro-ara-5-fluorocytidine, amylase concentration of the parotid glands of rats fasted for 18 hours prior to enzyme determination was hardly reduced from levels in unstimulated glands. Amylase activity of each parotid gland was 400, 438, and 600 mg/mg wet weight (three rats); after 3 hours of stimulation, there was no additional change and levels of amylase concentration were 419, 400, and 641 mg/mg. Levels for unstimulated glands were 440, 480, and 520 for the parotid glands (three rats). This is in marked contrast to the effects induced with cyclocytidine. Two hours of stimulation with 500 mg/kg of cyclocytidine reduced amylase concentration to levels one tenth of control levels (approximately 40-50 mg/mg).

Sialorrhea is therefore not a problem with the use of anhydro-ara-5-fluorocytidine and the drug appears, on the basis of measurements of amylase concentration, to be devoid of β-adrenergic action.

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[3]Cyclocytidine: CAS reg. No. 10212-25-6; *6H*-furo-[2′,3′:4,5]-oxazolo-[3,2-*a*]pyrimidine-2-methanol, 2,3,3*a*,9*a*-tetrahydro-3-hydroxy-6-imino-, monohydrochloride.

Anhydro-ara-5-fluorocytidine: CAS reg. No. 40505-45-1; O^2,2′-cyclocytidine, 5-fluoro-, monohydrochloride. Obtained through the courtesy of Dr. J.A.R. Mead, DCT, NCI, and the Drug Development Branch, Drug Research and Development, DCT, NCI.

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Treatment of Solid Tumors and Lymphomas With Methyl-CCNU (NSC-95441): A Phase II Study[1,2]

Dincer Firat and Gulten Tekuzman[3,4]

Brief Reports and Preliminary Communications

Methyl-CCNU was administered orally to 112 patients with proven, advanced, or recurrent carcinomas, soft tissue sarcomas, and lymphomas who had at least one measurable parameter of the disease and an estimated minimum survival of 2 months. There was a minimum 3-week treatment-free period for patients who had received prior chemotherapy or radiotherapy. The extent of the disease and the disease parameters to be followed were investigated by the appropriate clinical, radiologic, and radioisotopic examinations initially and at the end of the 4-6-week followup periods. Methyl-CCNU was given orally at a dose level of 200 mg/m^2 of body surface area in one dose every 6 weeks. The initial dose was adjusted up or down by 25 mg/m^2, depending on the blood cell counts prior to each course. Treatment was temporarily discontinued when leukocyte counts fell below 3000 cells/mm^3 and when platelet counts fell below 75,000 platelets/mm^3. A minimum of two courses of treatment was given before responses were evaluated unless rapid progression of the disease occurred. Patients with complete or partial objective responses continued to receive methyl-CCNU treatments until relapse or prohibitive toxicity occurred. The 25%-50%, 50%-100%, and 100% regressions of the sum of the products of the diameters of all measurable lesions were considered to be partial-fair (PF), partial-good (PG), or complete responses (CR), respectively. For the patients with primary brain tumors, improvement of the neurologic functions and a greater than 50% reduction of the papilledema or tumor mass as determined by brain scans were considered an objective response. When corticosteroids were administered, the dose was reduced to a minimum prior to methyl-CCNU treatments and was not increased until responses were evaluated.

RESULTS

There were 72 men and 40 women (median age 44 years) with various solid tumors, sarcomas, primary brain tumors, and lymphomas included in the study (table 1). Ninety-two patients were evaluable; the others were either lost to followup (eight patients) or died of their disease within 4 weeks of treatment (12 patients).

Only ten of the 64 evaluable patients with solid tumors and sarcomas (brain tumors and lymphomas excluded) showed objective responses that lasted 2-8 months (median 4 months). These remissions were partial, with the exception of one complete remission that occurred in a patient with sweat gland adenocarcinoma. The testicular tumors included two seminomas and four carcinomas. Among seven evaluable patients with lung cancer, there were four with epidermoid carcinoma (one partial response), two with anaplastic carcinoma, and one with giant cell carcinoma.

The primary brain tumors, which included five malignant glioblastomas and nine astrocytomas grade 2-3, were among the most responsive tumors. Ten of the 12 evaluable patients had previously received radiotherapy at a tumor dose of 4500-6000 rads delivered by a radiation source of cobalt 60 over a period of 5-6 weeks, in addition to the surgical excision or aspiration of the tumor. The diagnosis was based on clinical findings in two other patients. The majority of the patients were receiving dexamethasone[5] (2-6 mg/day, orally), and neurologic signs were either stable or progressive prior to the methyl-CCNU therapy. The radiotherapy preceded the chemotherapy by 1.5-17 months (median 6 months) in ten of the 12 evaluable patients. In two other patients, methyl-CCNU was given without irradiation. Although there were eight objective remissions (table 1), in general the neurologic deficits worsened within 4-8 months (median 5 months) and were responsive to the higher doses of corticosteroids in some patients. The methyl-CCNU treatments were continued in all patients despite no response or relapse in order to observe the effect of the

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[2]Methyl-CCNU: CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute.

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[5]Dexamethasone: NSC-34521; CAS reg. No. 50-02-2; pregna-1,4-diene-3,20-dione, 9-fluoro-11β, 17,21-trihydroxy-16 α-methyl-.

TABLE 1.—Phase II study of methyl-CCNU in patients with solid tumors, sarcomas, and lymphomas

Type or site of malignancy	Total No. of patients	No. of evaluable patients	No. of patients responding	No., type, and duration of responses (mos)
Head and neck	17	14	2	2 PF (5,4)
Gastrointestinal	10	8	0	—
Lung	8	7	1	PF (2)
Ovary	8	6	1	PG (5+)
Melanoma	6	5	2	2 PF (2,2)
Soft tissue sarcoma	6	5	1	PG (4+)
Testis	6	5	0	—
Osteogenic sarcoma	5	4	1	PG (8+)
Cervix	2	2	0	—
Sweat gland	2	2	2	CR (7), PF (3)
Miscellaneous	8	6	0	—
Primary brain	14	12	8	CR (4), 6 PG (2-6+), PF (8)
Hodgkin's disease	7	6	5	CR (9+), 2 PG (3+,5), 2 PF (3+,7)
Lymphosarcoma	5	4	1	PF (12+)
Reticulum cell sarcoma	8	6	2	PG (2), PF (3+)
Total	112	92	26	3 CR, 11 PG, 12 PF

treatments on the overall survival time of the patients with primary brain tumors. The median survival from the time of diagnosis of seven patients who died of their disease was 18 months (range 7-28 months).

The methyl-CCNU treatment induced remissions in one half of the 16 evaluable patients with lymphomas. All patients with stage II disease (seven patients) had previously received extended-field irradiation (3500-4000 rads) and showed recurrences within radiation ports prior to the chemotherapy. One half of the patients (stages II-IV) were resistant to the prior combination chemotherapy. The average duration of the remissions was 4 months (range 2-12 months); five patients are still in remission.

The major toxic effects of the methyl-CCNU treatments consisted of nausea and vomiting within 6-8 hours in 75% of the patients, leukopenia (less than 4000 cells/mm^3) in 52% of the patients, and thrombocytopenia (less than 100,000 platelets/mm^3) in 11% of the patients, usually in the 4th week. Anemia seldom occurred (nine of 92 patients). In the large majority of patients, recovery occurred in the 6th week. There were no drug-related deaths.

DISCUSSION

The nitrosourea compounds appear to be a promising group of alkylating agents that are effective against various neoplasms in man.[6] The effectiveness of BCNU[7] in the treatment of acute leukemia, multiple myeloma, various solid tumors, and lymphomas, as a single agent or in combination with other drugs, has already been demonstrated (1). These compounds possess all the necessary characteristics for crossing into brain and cerebrospinal fluid (2). The remissions of patients with primary brain tumors and meningeal leukemias who receive BCNU and CCNU[8] have been reported (3,4). Methyl-CCNU, although more lipid soluble, penetrates the blood-brain barrier somewhat less than CCNU, but it is the most effective agent among the nitrosoureas against a variety of tumors in animals.[6]

Although the experience with methyl-CCNU is not extensive, initial studies indicate its effectiveness in the treatment of lymphomas, malignant melanoma, and primary brain tumors (5). Our findings are consistent with the previous reports that methyl-CCNU has a limited use in the treatment of bronchogenic carcinoma (6). Only one of the four patients with epidermoid carcinoma in our series showed a temporary partial response. There were no responses seen

[6]Comis RL, Broder LE, and Carter SK. Clinical brochure: 1-(2-chloroethyl)-3-(4-methyl-cyclohexyl)-1 nitrosourea (Me-CCNU), NSC 95441, Aug 1972. Prepared by the NCI, Bethesda, Md.

[7]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

[8]CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

in two other patients with anaplastic and one patient with giant cell carcinoma of the lung. One complete and one partial remission that occurred in patients with sweat gland carcinoma (which is generally resistant to chemotherapy) are interesting and deserve further studies. Methyl-CCNU appears to be highly effective in the treatment of primary brain tumors. Although the number of patients is not sufficient, our response rate (eight of 12 patients) in primary brain tumors is above that which has been reported with CCNU (4). The overall survival of the patients may be a good criterion in evaluating the effectiveness of the chemotherapy in primary brain tumor, since the assessment of the degree of the neurologic deficit as a criterion of response can be difficult and may be influenced by many other factors. The median survival time (18 months) of our seven patients (four with astrocytomas and three with glioblastomas) seems to be higher than what would be expected without the use of chemotherapy (7).

In our patients with lymphomas, methyl-CCNU appears to be more effective in treating Hodgkin's disease. The disease, however, was less advanced (five patients with stage II disease), and the methyl-CCNU treatment was given prior to quadruple chemotherapy in most of these patients. The overall response rate suggests that methyl-CCNU is at least as effective as its parent compound, CCNU, in the treatment of malignant lymphomas (8).

In general, the responses induced by methyl-CCNU are of short duration and may require a combination of other effective agents for maintenance therapy. The toxic effects of methyl-CCNU are similar to those of other nitrosoureas and mainly consist of acute nausea, vomiting, and reversible myelosuppression within 4-5 weeks. At the dose level and with the schedule used here, complete recovery usually occurred within 6 weeks.

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5-Fluorouracil (NSC-19893) Compared to Cyclophosphamide (NSC-26271) in Bronchogenic Carcinoma: Results of a Clinical Study[1,2]

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According to the collected data of Livingston and Carter (1), the overall response rate to 5-fluorouracil (5-FU) in lung cancer was 6% of 143 evaluable patients from 17 literature reports. However, information regarding the tumor cell type was not recorded (2). A randomized, controlled clinical trial to compare cyclophosphamide to 5-FU was designed, and the results of the study are presented.

METHODS

Study Design

Eligible patients, stratified according to the tumor cell type, were randomly selected to receive 5-FU or cyclophosphamide. Whenever possible, patients who showed progression of disease received the alternate drug in a cross-resistance design.

5-FU was given intravenously (iv) as an initial "loading" course of 600 mg/m^2 in 250 ml of saline solution over a period of 3 hours, daily for 5 days every 2 weeks. No upward dose modification was done. Cyclophosphamide was given according to the standard dose level and schedule which consisted of 1.1 mg/m^2, iv, every 3 weeks.

Patient selection factors included: histologic diagnosis of lung cancer (3); locally advanced and disseminated disease not treated by conventional methods; performance status over 40% on the Karnofsky scale; presence of measurable disease; no prior chemotherapy; and the ability to continue followup in the outpatient clinic.

Results were classified as complete response, partial response (over 50% decrease in tumor size), and progression.

[1]Received Dec 9, 1974; revised June 19, 1975; accepted July 22, 1975.

[2]5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU.
Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

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[4]The authors wish to acknowledge the support and collaboration of the staff and residents of the Hospital General de Asturias.

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RESULTS

From August 1973 until May 1974, 65 consecutive eligible patients who had been referred to the Hospital General de Asturias entered the study. Cyclophosphamide was given to 32 patients and 5-FU was given to 33 patients. Four of the 65 patients (two from each group) were lost to followup and are excluded from the final analysis.

The clinical characteristics of the patients receiving either drug were as follows. All of the patients in the cyclophosphamide group were men. Their ages ranged from 44 to 75 years (mean 58.03 years). Thirteen patients had locally advanced disease and 19 had dissemination. Ten patients had had previous surgical or radiation therapy.

In the 5-FU group, there were 32 men and one woman. Their ages ranged from 47 to 76 years (mean 59.65 years). Sixteen patients had locally advanced disease and 17 had dissemination. Eleven patients had had previous surgical or radiation therapy.

Response

No complete responses were observed (table 1). Among the 30 evaluable patients receiving cyclophosphamide in the first part of the study, two showed partial response (PR). Of the 11 patients receiving cyclophosphamide in the cross-resistance phase, two had PRs. Thus, the total response rate for cyclophosphamide was 9.75% (four of 41 patients). Similarly, among 31 evaluable patients receiving 5-FU in the first part of the study, two achieved PRs. Of nine patients receiving 5-FU in the cross-resistance phase, one had a PR. The total response rate for 5-FU was 7.5% (three of 40 patients).

Results According to Cell Type

In the epidermoid category, three of 17 patients who received cyclophosphamide had PRs (17.64%). In the same category, three of 21 patients who received 5-FU demonstrated PRs (14.23%).

In the small-cell category, only one of six patients receiving cyclophosphamide showed PR. No responses were observed among six patients receiving 5-FU.

In the category of anaplastic large-cell carcinoma, no responses were observed in 17 patients re-

TABLE 1.—Distribution of patients according to tumor-cell type, chemotherapy given, and response to therapy*

Chemotherapy given	Total No. of patients	Distribution of responses according to cell type (No. of patients/No. of PR)			
		Epidermoid	Oat cell	Adenocarcinoma	Anaplastic large-cell
Cyclophosphamide, initially	30	14/2	4/0	2/0	10/0
Cyclophosphamide, crossover	11	3/1	2/1	—	6/0
Total	41	17/3	6/1	2/0	16/0
5-FU, initially	31	15/2	5/0	1/0	10/0
5-FU, crossover	9	6/1	1/0	1/0	1/0
Total	40	21/3	6/0	2/0	11/0

*Two patients from each group were lost to followup and were not included in this final analysis. See Results in the text.

ceiving cyclophosphamide, or in 12 patients receiving 5-FU. As for the category of adenocarcinoma, no responses were seen in four patients (two from each treatment group).

Survival and response times are tabulated in table 2. At the time of this report, all patients who had shown PRs had progressive disease.

TABLE 2.—Survival and response times

	Time given in days		
	Median	Mean	Range
Progression			
Total survival time for—			
Cyclophosphamide group	60	85	15-350
5-FU group	80.5	110	10-545
All patients	73	98	10-545
Partial response			
Start of response	21	16.2	7-42
Duration of response	160	199.4	80-420
Total survival time for all patients	217.5	285.6	140-451

Drug Toxicity

No severe or life-threatening toxicity was observed. Two patients who received cyclophosphamide and four patients who received 5-FU experienced dose-limiting hematologic toxic effects. Acute gastrointestinal toxicity and (rarely) mucositis and cystitis required symptomatic therapy, but in no instance did these effects require downward drug-dose modification. No other organ toxicity was identified other than alopecia after cyclophosphamide administration. Prophylactic antibiotics were administered intermittently to patients in whom pulmonary infection was a high risk.

CONCLUSIONS

Data from this study confirm the overall minimal activity of 5-FU in the treatment of lung cancer. Epidermoid carcinoma is slightly sensitive to 5-FU, while anaplastic large-cell carcinoma is resistant to 5-FU. Oat cell carcinoma and adenocarcinoma were not properly evaluated to draw conclusions. Activities of 5-FU and cyclophosphamide appeared comparable with regard to response and survival time of patients. Cyclophosphamide was minimally active in epidermoid carcinoma and was not active in anaplastic large-cell carcinoma. Short, intermittent courses of 5-FU appeared to be well tolerated with mild toxicity. Of epidemiologic interest is that so far, the incidence of lung cancer in Northern Spain and the response to chemotherapy appears to be similar to the incidence and response of lung cancer to chemotherapy elsewhere. The standard therapy control with cyclophosphamide established by this study is being used as a reference for new studies.

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Clinical Trial of the Oral Form of a New Podophyllotoxin Derivative, VP-16-213 (NSC-141540), in Patients With Advanced Neoplastic Disease[1,2]

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VP-16-213 is a semisynthetic podophyllotoxin derivative which has shown remarkably high antitumor activity in experimental animal systems (1,2) and has a moderate antineoplastic effect when given intravenously (iv) to patients. When an oral form of VP-16-213 became available, the present study was initiated to determine the optimal dose.

MATERIAL AND METHODS

The starting dose was 120 mg/m^2 administered orally each day for 5 days, repeated at 10-15-day intervals. This dose was selected on the basis of (*a*) the reported intestinal absorption of about 50% of an oral dose (Sandoz, Basle, personal communication); (*b*) the knowledge of a maximum tolerated dose of 45 mg/m^2 with iv administration daily for 7 days (3); and (*c*) the demonstration that intermittent treatment is superior to continuous treatment in animal tumors (1,2).

VP-16-213 was supplied in 100-mg capsules and the dose levels tested were approximations to 120, 180, 240, and 300 mg/m^2/day.

At the highest dose level, gastrointestinal side effects necessitated division of the daily dose into two. The planned length of treatment was at least 10 weeks or until progression of disease.

All patients had microscopically confirmed neoplastic disease not amenable to conventional treatment. The diagnoses were: cancer of the lung, 20 patients; genitourinary cancer, 12; gastrointestinal cancer, ten; cancer of the breast, eight; malignant lymphomas, five; acute leukemia, five; soft tissue sarcomas, five; melanoma, four; and pseudomyxoma, one. Hematologic requirements for entry into the study were a white blood cell count ≥ 3500 cells/μl and a platelet count ≥ 100,000 platelets/μl, unless low counts were disease-related. Furthermore, normal renal and hepatic function was required.

RESULTS

Seventy patients received a total of 255 5-day courses of therapy (median, three; range, one to 11). Eight patients were treated at two dose levels. Eighteen patients received only one course of treatment due to rapid progression of their disease.

Clinical Tolerance

VP-16-213 produced three types of side effects: hematologic toxicity, gastrointestinal toxicity, and alopecia.

Hematologic toxic effects.—These consisted mainly of leukopenia (42% of all patients) and thrombocytopenia (9%). No correlation was observed between the dose level and the frequency or severity of the hematologic toxic effects in comparable groups of patients. In patients who had received three or more series of prior therapy, the frequency of leukopenia (19 of 28 patients; 68%) was significantly higher ($P < 0.005$, Chi^2 test) than in those who had received no prior therapy or fewer than three series of prior therapy (14 of 50 patients; 28%) (table 1). In the first 5-day series, the nadir of leukopenia occurred on Days 10-12 and subsided on Days 15-21. A 10-day interval between the series seemed safe, and with the schedule employed, no cumulative toxicity seemed to develop.

Gastrointestinal toxic effects.—Nausea and vomiting occurred 2-6 hours after drug intake. The frequency of this side effect increased from 33% in the lower-dose group to 71% in the highest-dose group (table 1), a fact which made investigation of higher dose levels difficult.

Alopecia.—The frequency of alopecia was 13% in the 120-mg/m^2 group and 43% in the highest-dose group (table 1).

[1]Received Jan 17, 1975; revised May 30, 1975; accepted June 23, 1975.

[2]VP-16-213: 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside). Supplied by Sandoz, Ltd., Basle, Switzerland.

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TABLE 1.—Side effects during treatment with VP-16-213

Dose (mg/m²/day)	Total No. of patients	No. of patients with—		No. of patients with—†													
				Leukopenia				Thrombocytopenia				Gastrointestinal toxic effects			Alopecia		
		Minimal prior treatment	Major prior treatment	I	II	III	IV	I	II	III	IV	I	II	III	I	II	III
120	30	15		3	1				1			4	1	1	1		1
			15	2	3	2	1		1	1		2	2		1	1	
180	16	11		3	1							1	3	2		3	
			5	2	2			1					1		1		
240	18	14		2	2	1						4		4		1	3
			4		2		1	1		1			1				1
300	14	10		1								3	4	1			5
			4	1	2	1			1			2					1

*Minimal prior treatment = < three series of chemotherapy, radiotherapy, or both; major prior treatment = three or more series of chemotherapy, radiotherapy, or both.

†*Grade of severity*

Leukopenia: I = > 3000-4000 cells/μl; II = > 2000-3000 cells/μl; III = $\geq$ 1000-2000 cells/μl; IV = < 1000 cells/μl.
Thrombocytopenia: I = > 75,000-100,000 platelets/μl; II = > 50,000-75,000 platelets/μl; III = $\geq$ 25,000-50,000 platelets/μl; IV = < 25,000 platelets/μl.
Gastrointestinal toxic effects: I = mild; II = moderate; III = severe.
Alopecia: I = mild; II = moderate; III = severe.

No significant changes were observed in other nontumor-related clinical or biochemical parameters measured.

Antineoplastic Effect

In this phase I study, only a fraction of the patients were treated until hematologic toxic effects occurred, and due to the gastrointestinal toxicity, we could not study dose levels above 300 mg/m².

In five patients, partial or complete remission was obtained. One patient with metastatic cancer of the breast received courses at a dose level of 120 mg/m². After the sixth course, a 12 × 15-cm large subcutaneous metastasis and a 2.5 × 3-cm axillary node disappeared. She was still in complete remission 10 weeks later when she refused further treatment. She did not experience hematologic toxic effects.

The partial remissions were of rather short duration and were seen in one patient with a metastatic Wilms' tumor (3 weeks, 120 mg/m²), one patient with a recurrent liposarcoma of the abdomen (4 weeks, 120 mg/m²), one patient with a disseminated reticulum cell sarcoma (3 weeks, 180 mg/m²), and one patient with pulmonary metastases from a sarcoma of the uterus (11 weeks, 240 mg/m²). All four patients had received several series of prior chemotherapy, and all experienced hematologic toxic effects (degrees II, III, I, and IV, respectively) during the treatment (table 1).

DISCUSSION

In animals, VP-16-213 has shown a definite dose-dependent toxic effect and an antineoplastic effect when given iv or intraperitoneally (1,2). In man, iv treatment with VP-16-213 leads to hematologic toxicity which is also dose-dependent (3). The preliminary reports of a gastrointestinal absorption of VP-16-213 of 47% $\pm$ 4% in man (Sandoz, Basle, personal communication) were promising and seemed a sufficient basis for a phase I clinical study of the oral compound. The results reported here, however, are not compatible with a relatively constant absorption and/or inactivation because dose-dependent hematologic toxicity in groups of patients comparable with respect to prior treatment could not be demonstrated over as wide a range as 120-300 mg/m². Methods for measuring blood levels of VP-16-213 unfortunately were not available.

The evaluation of antitumor effect after oral administration of VP-16-213 is therefore difficult

since the low order of activity might or might not be due to insufficient absorption and/or inactivation. This problem is evident in the evaluation of the EORTC trial of VP-16-213 (4) in which 200 patients received iv treatment and 50 patients received oral treatment at a very low dose (100 mg/m²/day for 5 days). The data were pooled, but it is not mentioned whether the responders had received iv or oral treatment. In another study of oral VP-16-213, reported in abstract form (5), partial remission was seen in eight of 72 patients with neoplastic disease who received doses of 300-400 mg/day for 5 days with 9-day rest intervals between courses. Hematologic toxicity was not reported in detail.

Thus, although the oral administration of VP-16-213 does lead to some antineoplastic activity, the variability in absorption (or inactivation) and accordingly the unpredictable hematologic toxicity are definite arguments in favor of the continued use of iv administration. Further pharmacologic studies, especially of the absorption and metabolism of various galenic forms of the drug, are needed before more extensive trials of this oral compound can be undertaken.

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5-Fluorouracil (NSC-19893) Therapy for Pancreatic Carcinoma: Comparison of Oral and Intravenous Routes[1,2,3,4]

Brief Reports and Preliminary Communications

David C. Stolinsky, Reginald P. Pugh, and Joseph R. Bateman[5,6]

After more than a decade of extensive clinical use, 5-fluorouracil (5-FU) has become established as an antineoplastic agent, but one of limited value (1). In a previous report of the search for increased effectiveness (2) we compared the therapeutic and toxic effects of this agent when it was administered by oral and intravenous (iv) routes. That study included only five patients with pancreatic cancer, and no responses were obtained. An extension of this previous study, restricted to patients with pancreatic cancer, is described in the present report.

METHODS

Patient Selection

Patients with measurable pancreatic adenocarcinoma lesions that were not amenable to surgical or radiation treatment were eligible for the study. Patients who previously had been treated with cytotoxic agents or irradiation were eligible provided that at least 3 weeks had elapsed since cessation of the therapy. Patients who had received prior 5-FU therapy were ineligible for this study. Patients were required to have a pretreatment leukocyte count of at least 4000 cells/mm^3 and a serum creatinine level less than 1.8 mg/100 ml. No minimum performance status (3) was required. Each of the 30 patients entered into the study had a histologic diagnosis of carcinoma consistent with pancreatic origin; patients with islet cell carcinoma were not included. In 26 patients diagnosis was established by laparotomy with biopsy. The remaining four patients (two in the iv group and two in the oral group) had a presumptive diagnosis of pancreatic origin based upon celiac arteriography, suggestive upper gastrointestinal roentgenograms, elevated alkaline phosphatase levels, obstructive jaundice, and the absence of another primary site despite thorough evaluation.

Clinical Evaluation

The following pretreatment studies were performed: history and physical examination; complete blood cell count (cbc) including platelet count; urinalysis; determination of serum creatinine, bilirubin, alkaline phosphatase, and glutamic pyruvic transaminase levels; chest roentgenograms; and other roentgenograms and hepatic scintiscans when indicated. Physical examination and cbc were repeated weekly. Serum chemical determinations were repeated every 2-4 weeks, and roentgenograms and scintiscans were repeated when indicated. In the assessment of response, the products of the perpendicular diameters of each measurable lesion (palpable or scintigraphic) were added together. Response of hepatomegaly was determined by a 50% decrease in total craniocaudal span (percussion or scintigraphic) with a 50% fall in elevated serum bilirubin or alkaline phosphatase levels.

Of the 30 patients, 14 (seven in the iv group and seven in the oral group) had measurable abdominal masses. The remaining 16 patients (seven in the iv group and nine in the oral group) had hepatic abnormalities, including hepatomegaly in 12 and abnormal liver function tests in all 16. Statistical calcula-

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[2]Supported in part by Public Health Service grant CA-05186 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU. Supplied by Drug Research and Development, Division of Cancer Treatment, NCI, for intravenous use and purchased from commercial sources for oral use.

[4]Presented in part at the annual meeting of the American Society of Clinical Oncology, San Diego, Calif, May 7, 1975.

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tions utilized Fisher's exact test for comparison of the two response proportions (4).

Drug Administration

Patients were randomly assigned to either the oral or iv route of administration. For both groups the 5-FU dose was 15 mg/kg once per week. The drug given iv was administered in a single injection; the oral drug was the same material diluted in tap water. If neither response nor toxic effects were observed after four weekly doses, the dose was increased to 20 mg/kg for the next two doses. If no response was achieved after six weekly doses, the drug was stopped.

RESULTS

Thirty patients were entered into the study; 16 received 5-FU orally and 14 received 5-FU iv. All patients were evaluated for response and for toxic effects, although six died of their disease within 3 weeks after the start of 5-FU therapy. Only two of the patients had received prior chemotherapy. The median pretreatment performance status (3) was 70 for both the oral and iv groups (range 30-90 for both groups). The median pretreatment serum bilirubin level was 0.6 mg/100 ml for the oral group and 1.0 for the iv group. Three patients in the oral group and four in the iv group had pretreatment serum bilirubin levels greater than 2.0 mg/100 ml.

Response

A partial response was defined as at least a 50% reduction in the size of measurable lesions together with sustained or improved performance status for at least 1 month. A minimal response was defined as a 25%-49% reduction in the size of measurable lesions for at least 1 month. Partial responses occurred in three patients who received 5-FU iv and in none who received 5-FU orally. A minimal response occurred in one patient who received 5-FU iv and in none who received 5-FU orally. The median duration of response was 3 months (range 2-4 months). Lesions that responded to therapy included hepatic and abdominal masses.

The median survival time from diagnosis was 3.5 months for the oral group and 5.5 months for the iv group. The median survival time for partial responders was identical to that of nonresponders in the iv group (5.5 months). The median survival time from the start of 5-FU therapy was 53 days for the oral group and 110 days for the iv group.

Toxic Effects

A gastric ulcer developed in one patient who received 5-FU orally. The ulceration healed when 5-FU was interrupted and did not recur when 5-FU was given via the iv route. The ulceration may have been related to therapy although this complication was not noted in any of the 55 patients in our previous study who received 5-FU orally (2). No stomatitis was observed in the current study. Table 1 reports the toxic effects encountered and the total number of patients in both the oral and iv groups who had toxic manifestations.

TABLE 1.—Toxic effects in patients with pancreatic cancer who received 5-FU once per week

	No. of patients treated by—	
Toxic effect	Oral route (16 patients)	Iv route (14 patients)
Leukopenia		
3000-3900 cells/mm³	5	1
2000-2900 cells/mm³	—	1
Thrombocytopenia		
100,000-148,000 platelets/mm³	2	1
60,000-99,000 platelets/mm³	1	1
Nausea		
Mild	3	1
Severe	2	1
Diarrhea	3	1
Alopecia	1	1
Neurologic	1	2

DISCUSSION

The overall response rate (partial plus minimal) was significantly higher ($P = 0.04$) for the iv group than for the oral group. The response rate for the iv group (21%) was in accord with past experience of others and ourselves with 5-FU given iv using various schedules in the treatment of pancreatic cancer (23% responses among 125 patients) (1). On the basis of the current response and survival data, oral 5-FU given weekly cannot be recommended as therapy for pancreatic cancer.

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Alternating Administration of Adriamycin (NSC-123127) and Vincristine (NSC-67574)-Actinomycin D (NSC-3053) in Advanced Sarcomas[1,2]

Brief Reports and Preliminary Communications

H. O. Douglass, Jr. and C. Karakousis[3,4]

SUMMARY

Fifteen patients with surgically incurable, advanced, and metastatic sarcomas were treated with courses of adriamycin and vincristine-actinomycin D alternating within a 7-week cycle. Three patients had objective partial responses for more than 3 months (liposarcoma, 4 months; fibrous histiocytoma, 15+ months; desmoid tumor, 19+ months) while two other patients (liposarcoma, leiomyosarcoma) had lesser responses. No beneficial effect could be attributed to therapy in two patients with leiomyosarcoma, two patients each with synovial cell sarcoma, fibrosarcoma, and chondrosarcoma, and one patient each with rhabdomyosarcoma and mesenchymoma. No additive effect of alternating full doses of these agents could be demonstrated over the published data on response to adriamycin or actinomycin D alone.

[Cancer Chemother Rep 59:1045-1047, 1975]

Until recently, the treatment of surgically incurable, recurrent, or metastatic sarcomas was generally unsatisfactory. With the exception of the embryonal and pleomorphic rhabdomyosarcoma, tumors of mesodermal origin are relatively resistant to radiation therapy and to many traditional chemotherapeutic programs. The response rates in "adult sarcomas" of 23% for actinomycin D (1) and 43% for vincristine combined with actinomycin D (2) have further stimulated investigations utilizing these agents. An interesting new agent is adriamycin, with objective response rates in sarcoma patients averaging 26% (3).

To date, the most promising combination has not included actinomycin D (4). This effective four-drug program, with responses in a wide range of sarcomas, resulted in a mean duration of response of 6 months. A logical parallel to this regimen would be the addition, instead, of adriamycin to vincristine-actinomycin D in an attempt to achieve an additive antitumor effect with a superior response rate.

METHODS

Patients with histologically confirmed, surgically incurable sarcomas (other than the more responsive forms of rhabdomyosarcoma) were treated, without randomization, on a regimen of actinomycin D and vincristine alternating with adriamycin, administered in a manner to supply full doses of all three agents.

Drugs were administered on a 7-week cycle with actinomycin D (10 μg/kg of body weight) being given intravenously (iv) daily on the first 5 days of the cycle. Vincristine (1.5 mg/m^2 of body surface area) was administered iv on Days 1 and 5. On Days 29, 30, and 31, the patient received adriamycin at a dose level of 25 mg/m^2 of body surface area, iv.

Three patients with leiomyosarcoma, two with synovial cell sarcoma, two with chondrosarcoma, two with liposarcoma, and two with fibrosarcoma were treated on this protocol. Also enlisted in this program were individuals with rhabdomyosarcoma, mesenchymoma, fibrous histiocytoma, and an unresectable progressing mesenteric desmoid fibromatosis. In 11 patients, two or more operative procedures had been performed in an unsuccessful attempt to surgically control the tumor. Five patients had been treated with radiation therapy, two patients had received chemotherapy, and one had received immunotherapy with tumor vaccine and bacillus Calmette-Guérin prior to treatment with the combination chemotherapy program. The indicator lesions included pulmonary metastases in seven patients,

[1]Received Mar 4, 1975; revised May 16, 1975; accepted May 22, 1975.

[2]Adriamycin: CAS reg. No. 23214-92-8; doxorubicin.
Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

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bone metastases in three patients, intra-abdominal masses in four patients, and an abdominal wall lesion in one patient.

RESULTS

Partial responses (defined as greater than 50% reductions in tumor size for a duration of more than 3 months) were seen in three patients: one patient with a liposarcoma, one with a fibrous histiocytoma, and the one patient with the desmoid tumor. The response of the patient with liposarcoma lasted 4 months; the duration of response of the patient with fibrous histiocytoma was 15 months; but the patient with the desmoid tumor remains a responder more than 22 months later. At the end of seven cycles of therapy, both of these latter patients had received a total dose of adriamycin of 525 mg/m^2 of body surface area. In subsequent cycles of therapy, the adriamycin has been omitted.

Two patients, one with a liposarcoma and the other with a leiomyosarcoma, showed transient responses of 2 and 3 months' duration, respectively, with total tumor reductions of less than 50%. The remaining ten patients showed progression of tumor growth.

The indicator lesions in patients responding to treatment were pulmonary metastases (two patients) and intra-abdominal or abdominal wall lesions (three patients). None of the three patients with bone metastases showed any beneficial result from treatment.

Mild hematologic toxicity (white blood cell count [wbc] nadir less than 4000 cells/mm^3) was seen in five patients; the lowest wbc recorded was 800 cells/mm^3 after the administration of adriamycin. There was no significant thrombocytopenia. Mucositis after actinomycin D and vincristine, in the form of oral and pharyngeal ulcerations and diarrhea, was severe enough in four patients to markedly limit nutritional intake for a week or more, but no patient had to be hospitalized.

DISCUSSION

The combination of two or more antineoplastic agents has proved to be successful in improving the rate of response to treatment in Hodgkin's disease and other lymphomas, leukemias, breast cancer, Wilms' tumor, and the rhabdomyosarcomas of childhood. When agents with similar toxicities are combined, either the dose of each component must be reduced or the drugs must be administered alternately, allowing recovery from the toxicity of one agent before the next drug is given. When there is evidence that the rate of response to one of the components of the chemotherapeutic regimen (ie, adriamycin) may be dose related (5), there is greater reason to utilize an alternating drug regimen. Such was the rationale for the therapy in this protocol.

More recent evidence from an experimental model (6) would cast doubt on this reasoning. Miyakawa et al (6) demonstrated that adriamycin increased the average survival of mice with L1210 leukemia by 60%, and methotrexate[5] increased it by 85%. When utilized sequentially (adriamycin, then methotrexate), the effect was a synergistic prolongation of survival (185%) whereas when these drugs were given in an alternating pattern, the increased survival was less than additive (105% increase in an average duration of survival).

Although 33% of the patients in this series appeared to obtain some benefit from the drug program, the objective response rate of 20% is of the same order of magnitude as that reported for two of the component compounds (actinomycin D and adriamycin) used as single agents. Thus, no beneficial effect on the rate of response by this combination can be demonstrated.

Two patients are worthy of special comment. A 57-year-old woman who had histologically confirmed multiple pulmonary metastases of a fibrous histiocytoma, has had progressive diminution in the size of the metastases over a period of more than 15 months; all lesions, during the last course of therapy, measured less than 50% of their original size in each of their three diameters.

A desmoid fibromatosis of the mesentery of the small intestine developed in a 43-year-old man after a total colectomy for familial polyposis. A small bowel resection was performed but the tumor recurred, and on reexploration was found to involve the mesentery of the small intestine with stenosis of the lumen of the remaining bowel, with invasion of the retroperitoneum and abdominal wall. The patient was markedly cachectic. After more than 19 months of chemotherapy, he has gained more than 100 pounds and has no palpable abdominal mass. There is no narrowing of the intestinal lumen and no ileostomy dysfunction.

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[5]Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-{*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl}-.

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Reversible Penile Calcifications Associated With Bleomycin (NSC-125066)-Induced Pulmonary Toxicity[1]

Brief Reports and Preliminary Communications

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Bleomycin is an antineoplastic antibiotic associated with a wide range of toxic effects, including pulmonary and cutaneous toxic manifestations, mucositis, alopecia, pyrexia, anorexia, and nausea and vomiting (1,2). Ectopic calcification has not been reported in these large series of patients treated with this drug although sclerotic cutaneous changes sometimes produced by the antibiotic are pathologically similar to those of scleroderma (3). We have recently observed a patient with lymphoma in whom soft tissue calcifications paralleled the course of presumed bleomycin-induced pneumonitis, suggesting a possible relation to the administration of the drug.

CASE REPORT

A 55-year-old white man was in good health until the summer of 1973 when he noted fever, night sweats, and weight loss. Because of calcified hilar nodes and a positive tuberculin test, antituberculous medication was given, without effect. In November of 1973, further evaluation revealed that he had hypercalcemia, diffuse histiocytic lymphoma as shown from an inguinal node biopsy, and no evidence of active tuberculosis. Two months later the patient was admitted to the NCI where it was found that he had stage IIIB histiocytic lymphoma. Hypercalcemia persisted and a serum parathyroid hormone determination was at the lower limits of detectability of the assay. The results of bone marrow and liver biopsies were negative, as were skeletal roentgenograms and scans.

When a drug combination of cyclophosphamide, adriamycin, vincristine, bleomycin, and prednisone (4) was given to the patient his fever and hypercalcemia rapidly regressed.[5] Initial therapy was well tolerated by the patient, but in March 1974 a blood culture taken during a shaking chill after a blood transfusion was positive for pneumococcus. Four days later, rales were present at the right lung base, and a chest roentgenogram revealed pulmonary infiltrates. He was given oral penicillin after further multiple blood cultures were negative and bleomycin was withheld because of possible pulmonary toxicity. A total of 90 mg of bleomycin had been given to the patient.

Two weeks later the patient returned and was afebrile but had worsening dyspnea and radiographic infiltrates. Arterial hypoxemia was also present. Penicillin was discontinued and an open lung biopsy was performed. Bacterial, fungal, and mycobacterial cultures were unrevealing and no organisms were seen on routine and special stains. A pathologic examination revealed diffuse interstitial fibrosis and alveolar lining cell proliferation and atypia, findings which are compatible with bleomycin toxicity. Prednisone and oxygen were administered and the patient recovered after a stormy hospital course. At his discharge in May 1974, basilar rales and a diffuse interstitial process seen on his chest roentgenogram persisted. Chemotherapy was resumed (with bleomycin deleted) and the patient entered complete remission.

Shortly after his discharge, the patient's abdominal roentgenogram revealed calcifications beneath the symphysis pubis (fig 1) which on soft tissue films of the penis (fig 2) proved to be located within the sinusoids and about the periphery of the corpora cavernosa. Previous films documented the absence of such calcifications before treatment. The patient denied penile trauma or inflammation, but since discharge he had noted painless rigidity of the proximal third of the organ, without functional impairment. The pulmonary symptoms slowly improved and by July 1974 the steroids were withdrawn. A ^{67}gallium scan, which had shown a diffusely increased uptake over the lungs in March of 1974 (a finding compati-

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[5]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,-3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17-21-dihydroxy-.

FIGURE 1.—Coned-down film of the symphysis pubis demonstrating prominent ring-like calcification in the region of the penile corpora cavernosa.

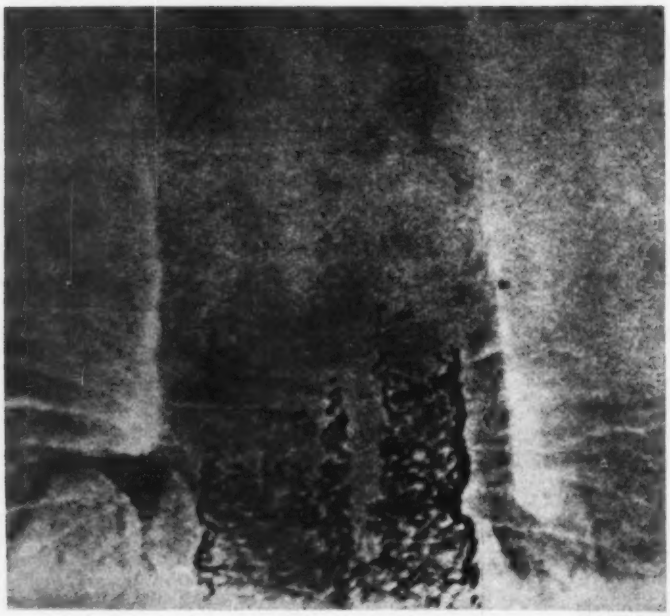

FIGURE 2.—Xeroradiograph of the base of the penis with fleck-like calcification within and about the periphery of the corpora cavernosa.

ble with bleomycin toxicity [5]), revealed a normal lung uptake in August.

An examination of the patient in November of 1974 showed that only a few basilar rales were present. Only a few chronic changes at the bases remained on his chest roentgenogram, and the penile calcification was almost totally resolved. Xeroradiographs taken at this time demonstrated only minimal calcium in the mid-portion of the penis and the proximal penile rigidity was gone. The patient's lymphoma remains in complete remission 12 months after the initiation of therapy.

DISCUSSION

Calcification in the human penis is a rare phenomenon. According to Joffe (6), it is usually secondary to local trauma or Peyronie's disease. This calcification varies in its location depending on the etiology. In Peyronie's disease, radiographs show bone formation involving the pectinate intercavernous septum with lateral and transverse prolongations (7). In the patient described by Joffe (6), the distribution about the corpora cavernosa produced a ring-like configuration on a roentgenogram. The radiographic appearance in our case is similar but, in addition, calcification was observed within the sinusoids of the corpora. Our patient did not experience trauma, and the distribution of the soft tissue calcium and its subsequent disappearance make Peyronie's disease unlikely.

Our patient exhibited the typical symptoms of bleomycin lung toxicity (1,2) with progressive dyspnea, basilar rales, and bilateral infiltrates and interstitial pneumonitis as seen on the roentgenogram. Microscopic changes are nonspecific but include alveolar cell proliferation and atypia, fibrinous exudate, and interstitial fibrosis (8). Toxicity is more frequent at cumulative doses over 450 mg, but there is an estimated incidence of 3%-5% in patients given substantially lower doses (1). Any role played by the other cytotoxic agents in modifying the pulmonary and possible soft tissue effects of bleomycin in this patient is unknown. Similarly, the contribution of hypercalcemia is unclear since the patient was normocalcemic at the time calcium deposition occurred.

This patient's penile calcification had such striking temporal association with the onset and resolution of his pulmonary toxic reactions that a common causation is strongly suspected. In an NCI autopsy series of patients with malignant neoplasms,[6] soft tissue calcification was found to be slightly more prevalent in patients treated with bleomycin than in those who were not. Soga et al (9) have found calcification in the resected tumors of Japanese patients with esophageal carcinoma who were given preoperative bleomycin. Although, to our knowledge, no other reported toxic manifestations of bleomycin have been accompanied by soft tissue calcification, our patient and the above observations suggest that this phenomenon could infrequently occur with the administration of the drug, especially in a setting of disordered calcium metabolism.

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Combination Therapy With 5-Azacytidine (NSC-102816) and Methyl-GAG (NSC-32946) in Previously Treated Adults With Acute Nonlymphocytic Leukemia[1,2]

John A. Levi and Peter H. Wiernik[3,4]

Despite the relative successes of daunorubicin and cytosine arabinoside individually and in various combinations as induction therapy for adults with acute nonlymphocytic leukemia (ANLL) (1,2), there is still a dearth of active agents for both induction and maintenance in this particular disease.

5-Azacytidine, a pyrimidine nucleotide analog, has shown definite activity as induction therapy for ANLL (3,4). In a previous study utilizing this drug in courses of 200 mg/m^2/day × 5, we obtained five complete remissions among 18 adults with previously treated ANLL (5).

Methyl-GAG given in daily doses of 150-200 mg/m^2 was shown to be an agent with significant activity in ANLL during the mid-1960s (6,7). However, because of quite severe toxicity (particularly gastrointestinal and mucosal toxicity) and a narrow therapeutic index, this drug is no longer in general use as a first-line induction agent. In previous reports methyl-GAG was given by daily short-term intravenous (iv) infusions to toxicity or response, but Venditti et al have shown that in mice bearing ascites L1210 leukemia an improved therapeutic index was achieved by administering the drug in intraperitoneal injections, each daily dose being divided into three hourly injections and repeated at 4-day intervals.[5]

Because of the proven activity of these two agents and their lack of cross resistance with other drugs being used for the treatment of ANLL, this current study was undertaken to (*a*) determine whether the combination could improve on the results achieved with 5-azacytidine alone and (*b*) whether administration of methyl-GAG in a continuous iv infusion would reduce the incidence of serious toxicity.

METHODS

Included in this study were eight adults with ANLL. All had received intensive prior therapy; four patients had never obtained a remission while the other four had had previous complete remissions but were now resistant to their initial therapies. No patient had previously received 5-azacytidine or methyl-GAG either as induction or maintenance therapy. Re-induction was attempted with 5-azacytidine at an initial dose of 100 mg/m^2/day by iv injection over 10-15 minutes in three divided doses for 5 days, and methyl-GAG at a dose of 100 mg/m^2/day by continuous iv infusion over 5 days.

Throughout induction, patients were examined daily, complete hemograms were performed five times a week, and liver and renal function and other biochemical tests were performed at least twice a week. Bone marrow examinations were performed at approximately 5-day intervals after each course of therapy.

Subsequent courses of therapy were given depending on the bone marrow response. Doses of each drug were increased by 50 mg/m^2/day if the marrow remained hypercellular with persistence of leukemic cells. Courses of therapy were repeated until remission was achieved, extreme toxicity occurred, or the patient demonstrated progressive disease. Complete or partial remission was diagnosed according to previously published criteria (8).

Full supportive care was given to all patients, including vigorous empiric or specific parenteral antibiotic therapy as indicated, prophylactic oral non-absorbable antibiotics, and platelet transfusions as previously described (5).

RESULTS

Data on the responses to this combination therapy are given in table 1. Only one patient achieved a partial remission on two occasions. Each response required two courses and the times to remission were 71 and 77 days. Durations of these unmaintained responses were short: 30 and 21 days. No other patient approached remission status although patient

[1]Received Apr 17, 1975; revised May 21, 1975; accepted May 27, 1975.

[2]5-Azacytidine: CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-.

Methyl-GAG: CAS reg. No. 24968-67-0; guanidine, 1,1′-[(methylethanediylidene)dinitrilo]di-, dihydrochloride, monohydrate; methylglyoxal-bis(guanylhydrazone).

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TABLE 1.—Results of therapy with 5-azacytidine and methyl-GAG

Patient No.	Sex, age	Diagnosis*	Previous therapy†	5-Azacytidine/methyl-GAG therapy			
				No. of courses	Dose (mg/m²)	Response‡	Survival§ (wks)
1	M, 67	AMML	D,A,T	1	100	NR	7
				1	150		
2	M, 59	AMML	A,T¶	1	100	NR	6
				1	150		
				1	200		
3	F, 46	AMoL	D,A,T¶	1	150	NR	5
				1	200		
4	F, 34	AMML	D,A,T	2	150	NR	7
5	M, 67	AMML	D,A¶	1	150	NR	2
6	F, 50	AML	D,A,T	1	150	NR	8
				1	200		
7	F, 55	AML	A,T	1	150	NR	16+
				1	200		
8	F, 25	AMML	D,A,T¶	a.1	100	PR	35+
				1	150		
				b.2	150	PR	

*AML = acute myeloblastic leukemia; AMML = acute myelomonocytic leukemia; AMoL = acute monocytic leukemia.
†D = daunorubicin; A = cytosine arabinoside; T = 6-thioguanine.
‡PR = partial remission; NR = no response.
§Survival from start of 5-azacytidine/methyl-GAG therapy.
¶Patients who failed to achieve remission with prior induction therapy.

No. 7 had definite bone marrow improvement of 31 days' duration. Survival from the start of therapy was brief for the remaining six patients who failed to respond to at least one course of treatment (2-8 weeks).

Courses of therapy at 100 mg/m² failed to produce marrow hypoplasia or significant antileukemic effect in the three patients treated at this dose. Courses of 150-200 mg/m² were more myelosuppressive and the median nadir white blood cell count after each course of therapy was 700 cells/mm³ (range, 200-1600). The median time taken to reach this nadir was 15 days (range, 5-30), with a median duration of 12 days (range, 4-25). Platelet count suppression was quite severe (median, 13,000 platelets/mm³; range, 4000-24,000) and followed a time course similar to that of the leukopenia.

All patients treated with this regimen suffered definite and often severe nonhematologic toxicity, particularly at the higher dose levels (table 2), Gastrointestinal disturbance, fever, and neuromuscular toxicity with myalgias, asthenia, and in one case progression to coma previously described after 5-azacytidine administration (5) were seen in all eight patients. However, the addition of methyl-GAG resulted in an increase in both the number and degree of severity of toxic effects. Mucosal toxicity including hemorrhagic pharyngitis, nasal ulceration, vulvovaginitis, and penile ulceration appeared to be directly related to methyl-GAG administration. Onset was generally 4-7 days after drug administration and lasted 7-10 days. This toxicity together with the frequently severe vomiting and diarrhea resulted in dehydration, infection, and renal dysfunction in four patients, two of whom died as a result. Fatal sepsis developed during the period of severe mucosal toxicity in two other patients who died with infection.

DISCUSSION

Based on our previous studies with 5-azacytidine as a single agent for reinduction of adult ANLL, it was hoped that the combination with methyl-GAG would result in a further improvement in therapeutic efficacy. Initial courses of this combination at 100 mg/m²/day × 5 were clearly inadequate and even

TABLE 2.—Nonhematologic toxicity

Toxicity	Severity of toxicity				
	Mild	Moderate	Severe	Extreme	Total
	(No. of patients)				
Nausea and vomiting	1	3	2*	2*	8
Diarrhea	1	2	2*	1*	6
Fever	2	5	1	0	8
Neuromuscular	1	3	3	1	8
Mucositis†	0	3	2	2	7
Local phlebitis	1	2	1	0	4
Renal dysfunction‡	0	0	2	2	4
Hepatic dysfunction	0	1	0	0	1

*Hemorrhagic in nature.

†Includes hemorrhagic pharyngitis; conjunctivitis; nasal ulceration; vulvovaginitis; urethritis and ulceration of the glans penis.

‡In each instance associated with dehydration and sepsis.

when doses of the drugs were increased to 150-200 mg/m²/day only one partial remission resulted. Since our previous experience had shown that 5-azacytidine given in courses of 200 mg/m²/day × 5 was effective (5), it is apparent that combining methyl-GAG with 5-azacytidine certainly added nothing to the therapeutic efficacy of the latter drug used alone and methyl-GAG may even have antagonistic properties, the mechanism being unknown. However, despite the relatively small number of patients included in this study, the failure to observe any significant responses even at the higher dose levels and the severe toxicity encountered precluded continued evaluation of this combination.

We were unable to confirm in a clinical setting the suggestive evidence from experimental animal studies that methyl-GAG would have an improved therapeutic index when given by continuous iv infusion. The mucosal toxicity in particular was frequently very severe and contributed directly to the deaths of four patients. Therefore, it appears that even with the further advances in supportive care now available and with the use of a different schedule of administration for methyl-GAG, this drug remains too toxic for general use in induction therapy for ANLL. 5-Azacytidine, however, must still be considered a drug of potential value and further combination studies with other agents are warranted.

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ANNOUNCEMENTS

Medical Oncology Fellowship

Applications are now being accepted for 2-year fellowships in medical oncology which will begin July 1, 1976, at the University of Southern California School of Medicine. The service comprises clinics with over 500 monthly visits and 59 beds. Fellows have responsibility for patient management and house staff teaching. Standard and investigational chemotherapy and immunotherapy will be carried out under the supervision of Dr. Joseph R. Bateman who is Chairman of the Western Cancer Study Group and Professor of Medicine. Support facilities include laminar air-flow isolator units, a continuous-flow cell separator, and on-site cytogenetics and immunology laboratories. Rotations include student ward, consultation service, and electives (eg, hematology, hematopathology, tumor surgery, gynecologic oncology, radiation therapy, or laboratory research). Applicants should have completed 2 years of medical residency and be eligible for a California license. The current salary is $19,284 for the first year.

Address inquiries to: Dr. David Stolinsky, 2826 So. Hope St, Los Angeles, Calif 90007.

Eighth World Congress of Gynecology and Obstetrics

Mexico City, October 17-22, 1976

The International Federation of Gynecology and Obstetrics announces the Eighth World Congress of Gynecology and Obstetrics to be held October 17-22, 1976, in Mexico City.

Only active members of national federations, societies, or colleges affiliated with the International Federation of Gynecology and Obstetrics may actively participate in this Congress.

There will be five plenary sessions, seminars on current topics of gynecology and obstetrics, correlated seminars, and three special invited lectures. Within this scientific program there will be free communications sessions, films, and precongress courses as well as scientific, technical, commercial, and artistic exhibits. In addition there will be a social program designed to enable attendants to become better acquainted.

The official languages of the Congress will be Spanish, English, and French with simultaneous translation into Spanish, English, French, and German for special lectures and plenary sessions.

Application forms to participate and to enroll in the Congress and further detailed information may be obtained from: Carlos MacGregor, MD, General Secretariat, Avenida Baja California 317, Mexico 11, D.F., Mexico.

Training Program in Medical Oncology

A 2-year training program in Medical Oncology is being offered at Tufts University School of Medicine and affiliated hospitals. Training is shared by the Medical Oncology units of the New England Medical Center, Lemuel Shattuck, and Pondville Hospitals. Elective rotations to Therapeutic Radiology, Surgical Oncology, Pathology, and Hematology are available. Fellowships require an internship and 1 or 2 years of previous residency training. The program is sponsored by the National Cancer Institute and carries a stipend of approximately $13,000 per year.

Further information may be obtained from: Larry Nathanson, MD, Chief, Medical Oncology Service, New England Medical Center Hospital, 171 Harrison Ave, Boston, Mass 02111.

Cancer Therapy Abstracts

The Franklin Institute Press announces the publication of Volume 16, No. 1 of *Cancer Therapy Abstracts* at a subscription price of $45.00 (domestic) and $55.00 (foreign).

Cancer Therapy Abstracts will include publications on chemotherapy and cancer-related surgery, radiotherapy, and immunotherapy. Volumes 16 and 17 will consist of 12 fully indexed issues plus cumulative annual author and subject (agents, human and experimental tumors, general subjects) indexes. The journal will contain approximately 6000 items per year. *Cancer Therapy Abstracts* computer tapes are also now available for an additional fee. Tapes of each issue will contain full citation, abstract or annotation, and index terms for each article.

To obtain *Cancer Therapy Abstracts* or the computer tapes please contact: Carl Martinson, The Franklin Institute Press, The Benjamin Franklin Parkway, Philadelphia, Pa 19103.

Postdoctoral Fellowships in Drug Development

These fellowships are now being offered at Sloan-Kettering Institute for Cancer Research, New York, New York, to postdoctoral candidates who have received their PhD or MD or equivalent degree at the inception of the fellowship. Fellows will be selected at various levels of postdoctoral experience not to exceed 5 years (except in unusual cases). Only United States citizens or foreign nationals with permanent resident or immigrant visas will be considered for these 1-year appointments.

Fellows will work in the development and evaluation of chemotherapeutic agents which act specifically or selectively against malignant growth. Training will include research in the chemical, biologic, and cellular aspects of abnormal as well as normal growth processes. It may also include broadly based fundamental studies involving several biomedical disciplines while other studies will be concerned directly with the control of neoplastic growth. Areas of concentration will include synthesis and isolation, preclinical drug evaluation, and mechanisms of drug action. Training methods will include laboratory work, individual research, lectures, seminars, and conferences.

For applications write: Dr. Dorris J. Hutchison, Coordinator-Field of Education, Sloan-Kettering Institute for Cancer Research, 410 East 68th St, New York, NY 10021. (An equal opportunity and Affirmative Action Employer)

Second Annual Meeting of the Association of Community Cancer Centers

Jacksonville, Florida, January 31 and February 1, 1976

The Association of Community Cancer Centers (ACCC) announces its second annual meeting to be held at the Hilton Hotel, 565 South Main St, Jacksonville, Florida (telephone: [904] 398-3561) on January 31 and February 1, 1976. This meeting is open to physicians, hospital administrators, nurses, rehabilitation specialists, and other persons interested in community cancer programs. Advanced reservations are recommended since the conference will be limited to 300 people. The registration fee is $50.00 for ACCC members and $75.00 for nonmembers.

For further information please contact: Marion Cernansky, Jacksonville Meeting, Association of Community Cancer Centers, P. O. Box 30279, Bethesda, Md 20014 (telephone: [301)656-3987).

Workshop on Antimetabolites and the Central Nervous System

New York, New York, February 2 and 3, 1976

This 2-day workshop will be held in the Kerbs Auditorium, 1275 York Ave, Rm 184-185 at Memorial Sloan-Kettering Cancer Center, New York, NY, on February 2 and 3, 1976. Its purpose is to enable scientists currently involved in the study of pharmacokinetics of methotrexate and other antifols, citrovorum factor, and cytosine arabinoside, to meet and discuss their work in order to better understand these compounds in relation to their use in the treatment of neoplastic disorders involving the central nervous system.

Co-directors of the workshop are Dr. William R. Shapiro, Department of Medicine, and Drs. Bipin M. Mehta and Dorris J. Hutchison, Laboratory of Drug Resistance and Cyto-Regulation. Registration is limited to 250 persons. For details of the program and preregistration information, write or call: Bipin M. Mehta, PhD, Walker Laboratory, Sloan-Kettering Institute for Cancer Research, 145 Boston Post Rd, Rye, NY 10580. Telephone: (914) OW 8-1100, Ext 251.

Seventh New Drug Seminar: Nitrosoureas

Washington, DC, December 15 and 16, 1975

The Division of Cancer Treatment, National Cancer Institute, is planning its Seventh New Drug Seminar for December 15 and 16, 1975, at the Washington Hilton Hotel. The subject will be the nitrosoureas. Two of these compounds (BCNU and CCNU) will soon be commercially available, and this conference will discuss their role in the therapy of cancer along with that of other drugs in this class.

As part of the Seminar, there will be two minisymposia on the treatment of the two major indications for the nitrosoureas: advanced gastrointestinal cancer and CNS neoplasms with emphasis on the malignant gliomas. This Seminar will be geared to the practicing oncologist and will attempt to place the nitrosoureas in their proper perspective within the clinical oncologist's armamentarium. The cochairmen of the Seminar will be Dr. Vincent T. DeVita, Jr. and Dr. Stephen K. Carter.

There will be no preregistration nor registration fee for this Seminar. For further information, please contact Dr. DeVita: Director, Division of Cancer Treatment, National Cancer Institute, Bldg 31, Rm 3A52, Bethesda, Md 20014.

Immunology Symposium on "Applied Clinical Immunology"

February 5-7, 1976

The University of Texas Health Science Center at Houston, Division of Continuing Education, in cooperation with Baylor College of Medicine and the University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, will present an immunology symposium on "Applied Clinical Immunology" on February 5-7, 1976.

A 2½-day course will be structured to present to the practicing physician and the laboratory scientist current methods and approaches used in clinical medicine. The principles of immunology on which laboratory tests and immune profiling are based will be reviewed. The rationale, interpretation, and details of commonly employed immune techniques (or those tests likely to be adopted in the near future) will be presented. The course will include discussion of radioimmunoassays, cell-mediated tests, antibody determinations, and certain methods assessing nonspecific immune factors. A final Saturday session will be devoted to laboratory demonstrations of many of the immune assays described.

For further information write: The Office of the Director, The University of Texas Health Science Center at Houston, Division of Continuing Education, PO Box 20367, Houston, Tex 77025.

Johananoff International Fellowship for Advanced Biomedical Studies

The Mario Negri Institute for Pharmacological Research of Milan, Italy, and the Mario Negri Institute Foundation, Inc, of New York invite applications for the 1976-1977 "Johananoff International Fellowship for Advanced Biomedical Studies" which was established through the generosity of Mr. S. Johananoff, a prominent international industrialist. The amount of the award is $15,000 for 1 year, including stipend and travel. There are no objections to the "Johananoff Fellow" retaining his/her regular stipend and other support.

This fellowship offers a distinguished scientist, internationally renowned for outstanding contributions in one of the following fields—cancer chemotherapy and/or immunology, cardiovascular pharmacology, neuropsychopharmacology, or drug metabolism—the opportunity to spend his/her sabbatical year at the Mario Negri Institute for Pharmacological Research in Milan. The task of this fellow will be to assemble, examine, and evaluate knowledge available in a well-defined area within his particular competence and to prepare a comprehensive review, containing a critical analysis of existing data and a synthesis of his thoughts and conclusions with suggestions for new directions of research. He/she will present a review of the "Johananoff Lecture" before an international scientific audience at the end of his/her stay in Milan. The full text of the review will be published as a separate monograph or as a paper in an appropriate scientific journal, for dissemination to the international biomedical community.

Eligible candidates should be other than Italian citizens, who are actively engaged in advanced research in one of the specialized fields mentioned above. Only scientists from academic or nonprofit institutions and government agencies will be considered. Applications for 1976-1977 should be airmailed no later than January 28, 1975, and must include: (1) curriculum vitae, (2) list of publications, (3) reprints of pertinent papers, and (4) an outline of the proposed study (about 250 words). The award will be announced by March 30, 1976, and its recipient may come to Milan any time thereafter within 1976.

Applications and/or requests for further information should be airmailed to: The Johananoff Fellowship Committee, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea, 62, 20157 Milan, Italy.

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